No. 29.

(NEW SERIES.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

PIROPLASMA CANIS AND ITS LIFE CYCLE IN THE TICK.

BY

CAPTAIN S. R. CHRISTOPHERS, M.B., I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.

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PIROPLASMA CANIS AND ITS LIFE CYCLE IN THE TICK.

I.

INTRODUCTION.

THE piroplasmata, apart from their economic importance, possess a special interest for the protozoologist. This interest centres round their morphology and life cycle, because the discovery of the nature of the developmental changes which they undergo in the tick promises to throw light on the relation of the hæmocytozoa to the flagellates, as well as to add greatly to our knowledge of the life processes of the pathogenic protozoa.

The subject of the present memoir is *Piroplasma canis*, which, although of little economic importance, has been chosen on account of its comparatively large size and because it is probable that what applies to it will be applicable, in the main, to other species of the genus. *Piroplasma canis* has lately been the subject of a comprehensive work by Nuttall and Graham Smith, but I have decided to describe it afresh in order that the complete life cycle of the parasite both in its mammalian host and in the tick may be dealt with in one work, and because even the asexual cycle of piroplasma requires more detailed study than it has yet received.

The piroplasmata first became known to science as a result of researches by the American workers Smith and Kilborne (120-122) who described under the name *Pyrosoma bigeminum* certain parasites of the red blood corpuscles of cattle which they showed to be the cause of the disease Texas fever. These observers fully recognised the protozoon nature of the parasites and their relationship to the malarial parasites of man and to certain other intracorpusoular parasites which shortly before had been observed in the blood of various lower animals. Before the date of publication of Smith and Kilborne's reports Babes (18) had seen the same organism in the blood of cattle suffering from hæmoglobinuria in Europe; but it seems that he did not recognise that it was a protozoon, since he named the organism *Hæmatococcus bovis* and considered at first tha he had grown it upon nutrient media.

Later work has shown that parasites closely resembling, if not identical with, those of Texas fever exist in the blood of cattle over almost all the tropical and subtropical world giving rise to the diseases variously known as Redwater,

Tristeza, etc. At present the question whether these represent one or more species is still an open one. Ligniéres, who described the South American forms, believes that more than one species is present in that country ; and the identity of the American and European parasites is not proved.

In East and South Africa, in addition to the large forms seen in Redwater, very minute bacillary forms were described by Koch (73) who considered them as forms of the Redwater parasite. They are now looked upon as a distinct species, *P. parvum* (Theiler) (127) the causal agent in a disease characterised by a series of definite morbid lesions and known under the names of Rhodesian Redwater, African Coast Fever or more usually East Coast Fever.

Similar parasites are also described as the cause of *tropical piroplasmosis* of cattle in Transcaucasus (42) and exist in the cattle of India (12) and of Japan (102).

In 1892 a species *P. ovis*, resembling in appearance *P. bigeminum*, was observed by Babes (136) in the blood of Roumanian sheep suffering from the disease known locally as Carceag, and similar parasites have been described as occurring in sheep in Italy (138), France (140*a*), Turkey (138), and South Africa (139).

Piroplasma canis was first described by Piana and Galli Valerio (161) in 1895, and malaria like parasites in the blood of the horse by Gugliemi (167) in 1899. The latter parasite, which has since been named by Laveran (1901) *P. equi* and differs in several respects from the typical piroplasmata, is the cause of biliary fever in horses, a disease which has been described as existing in Europe (167), Africa (168), and India (171*a*), and no doubt exists elsewhere. A very similar disease in donkeys and mules due to a piroplasma is described as being present at the Cape by Dale (164) who regards the parasite, though it has the general characters of *P. equi*, as specifically distinct. Piroplasmata in the blood of monkeys (Cercopithecus) in Africa are described but not named by Ross (183). Ziemann (183*a*) describes a species found in the Cameroons in the blood of the sheep, goat, horse and ass. Recently Fantham (177) has described a species (*P. muris*) in white rats obtained from a dealer in England.

What is probably a distinct species occurs in the sheep of Southern India. This is a minute form resembling, but still more minute than, *P. parvum*.

Probably other species as yet undiscovered also exist, the minute forms being especially likely to be overlooked * ; and the results obtained by Lounsbury with the tick *A. hebræum* certainly predisposes one to believe that the disease of

* Since writing the above a bacilliform species of piroplasma has been described by Bettencourt, Franca, and Borges in *Cervus dama* of Portugal (*T. Cervus*). These authors suggest the genus *Theileria* for those piroplasmata which adopt the bacillary form and divide to form four cocciform elements disposed in the form of a cross. Theiler has also described a species in cattle which he believes to be distinct (*P. mutans*).

sheep and other animals at the Cape known as "heartwater" is a piroplasmosis, in spite of the fact that no one has yet demonstrated any parasite in the blood of animals suffering from the disease.

The existence of a human piroplasmosis described by Anderson (176*a*) and Wilson and Chowning (183*b*) still remains unsubstantiated as does the presence of piroplasmata in the human subject described by Lingard and Jennings (180).

It is no longer possible to include the Leishman-Donovan parasite among piroplasmata, which I shall show do not pass through a flagellate stage in their development.

The inoculation of blood containing piroplasmata into animals of the species in which the parasite naturally occurs leads to infection in the case of most of the species. This was shown for the Texas fever parasite by Smith and Kilborne who observed the onset of symptoms and the appearance of parasites five or six days after the intravenous injection of defibrinated blood from sick animals. A similar fact in regard to *P. canis* was established by Spreul and Purvis (162*a*) and successful inoculations with *P. ovis* have been recorded by Motas.

In the case of *P. equi* considerable difficulty is found in transmitting the disease by inoculation even when large quantities of blood are used; but successful results have been obtained by Koch.

Inoculation with blood containing the parasite of East Coast fever has failed, in the hands of Koch and of Theiler, to give rise to infection and it would appear that this disease can be transmitted experimentally only by the bite of infected ticks. Inoculation of white rats and of monkeys with their respective piroplasmata has also so far failed.

An important point in transmission of the piroplasmata by inoculation is that the blood of recovered animals is infective even though parasites may not be demonstrable under the microscope. This was proved to be the case in Texas fever by Smith and Kilborne and also by Schroeder (117) who notes that the blood of recovered animals may be infective years after they have recovered from an attack.

Robertson (162) has shown the same in the case of "salted" dogs, their blood giving rise to fatal infection many months after recovery and at a time when by microscopical methods no parasites could be detected.

The injection of blood containing piroplasmata into animals of a species other than the normal host of the parasite has so far in no recorded case been followed by success. The piroplasmata thus differ from the pathogenic trypanosomata to most of which laboratory animals are susceptible. Smith and Kilborne injected large quantities of infective Texas fever blood into lambs, rabbits, guinea pigs, and pigeons, in each case without producing infection. Robertson

and Lounsbury (210) have found the jackal refractory to inoculation with the blood of dogs suffering from *P. canis* and to the bites of hundreds of highly infected ticks. With this species also Robertson notes failure to infect in the cases of the horse, ox, sheep, cat, rabbit, guinea pig, rat, mouse, and fowl; Nocard and Motas in the horse, ox, sheep, goat, cat, rabbit, guinea pig, white rat, white mouse, fowl, and pigeon, Nuttall and Graham Smith in the cat, ferret, hedgehog, guinea pig, and white rat. Monkeys (*Macacus*) and brown rats (*M. rattus*) inoculated by me with blood swarming with parasites have failed to show infection.

Normally, transmission of the piroplasmata takes place by ticks. That the infection of Texas fever was carried by ticks was conclusively shown by Smith and Kilborne. Even in the eighteenth century it had been recognised in America that apparently healthy cattle coming from a large but well-defined territory, including most of the Southern States, caused outbreaks of severe and fatal disease among healthy Northern cattle, and that Northern cattle proceeding into Southern territory were attacked with the same fatal malady. In the course of time many curious facts regarding this disease known as Texas or Redwater fever, came to light. Especially was it noticeable that it was not communicated directly from Southern to Northern cattle, but that the ground over which the former passed was infected by them and that the infection was transmitted thence to susceptible cattle. The early experiments of Smith and Kilborne pointed to the cattle ticks as in some way the cause of the disease and they were able to show that, when these ticks were artificially removed, Southern cattle could be placed with Northern cattle without ill effects. It was thought at first that the tick obtained the parasite from the blood of its host and that, on the dissolution of the tick on the pasture, a resistant spore form was set free which produced the disease when taken in with the food. But it became evident from several considerations that this could not be the case, and the fact that infection nearly always occurred at the time when the young ticks were first noticed on the animals, led Smith and Kilborne to feed larval ticks hatched in the laboratory upon oxen kept in stalls. In the first experiment three animals infected in this way died of Texas fever, and further experiments proved beyond all doubt that it was the bite of the young brood of infected Southern ticks which caused the disease. The source from which the mother ticks derived the infection was made evident when the blood of the apparently healthy Southern cattle injected into susceptible Northern cattle was found to give rise to the symptoms of Texas fever and to the presence of the parasite in the blood. The experiments of Smith and Kilborne were so convincing and the relation of ticks to infection so obvious when once demonstrated that it was not long before these results were confirmed by observations in other parts of the world.

But whilst Motas (213) showed that ovine piroplasmosis had been conveyed by ticks brought from an infected flock more than 200 kilometres distant, and Koch's researches upon Redwater still further demonstrated the connection of ticks with piroplasmosis, no very great advance in our knowledge of the subject occurred until Lounsbury and later Theiler undertook systematic researches upon the transmission of these diseases.

At the time when Smith and Kilborne first demonstrated the pathogenic properties of ticks knowledge regarding these pests was most meagre ; it was these authors who in conjunction with Curtice first brought accurate observation to bear upon their life history. They noted the hatching of minute six-legged larvæ and the development of these, after they had attached themselves to their host, through the nymphal to the adult stage. But they took notice of only a single species named by Curtice *Boophilus bovis* which they found on the infected Southern cattle.

The life history of other species has become known chiefly from the researches of Lounsbury, who made the discovery that all ticks had not the same life history ; the habit of passing through the metamorphosis attached to the skin of the same host not being universal or even usual among ticks, which in most cases drop from their host in order to undergo this change. Lounsbury also proved by a series of experiments lasting over three years that in South Africa piroplasmosis of the dog is conveyed by the tick *Hæmaphysalis leachi*, not by the larvæ from infected mother ticks, but only by ticks which, reared from eggs laid by infected mother ticks, had passed through two metamorphoses and had reached the adult stage.

The problem of transmission by ticks was still further complicated by the facts later demonstrated by this author and also by Theiler in regard to East Coast fever. In this case transmission by the bite of the progeny of ticks fed on sick animals always failed to infect ; but infection taken in by the larva was transmitted by the nymph and that taken in by the nymph was transmitted by the adult. The same method of transmission occurred in heartwater which was induced by the bite of nymphs or adults of the tick *Amblyomma hebræum* fed in a previous stage upon sick animals.

It is thus clear that the piroplasmata behave differently as regards the exac mechanism of their transmission, either in the case of different species of parasite, or when transmitted by different species of tick, two types of transmission being noticeable, the hereditary, and that taking place from one stage to another. Of the hereditary we have two forms, that seen in Texas fever where transmission is conveyed by the larva of the second generation, and that where the larva and nymph are both innocuous and infective properties develop in the

adult only. A third has to be added for I shall show that in the case of *R. sanguineus* nymphs of the second generation may be infective.

It is obvious that the explanation of these facts was difficult for, up to quite recently, when a few of the stages in development were described by Koch and Kleine, not a single trustworthy observation existed regarding the development of piroplasma in the tick. Even the work of Koch and Kleine left the nature of the changes undergone nearly as obscure as before, since the significance of the stages described by these authors was not at all clear and at most their observations formed only a few disconnected links in the chain of events.

II.

THE PARASITE IN ITS MAMMALIAN HOST.

Geographical Distribution.

Piroplasma canis was discovered by Piana and Galli Valerio in the blood of hunting dogs in Lombardy and described by them. Five years later Celli (32) noted its presence in hunting dogs brought from Lombardy to the Roman Campagna. In France the parasite is recorded by Leblanc (150) at Lyons and by Nocard and Almy (155) at D'Alfort, indigenous cases of infection in both instances being met with.

It has been described by Marchoux (151) as occurring in the blood of dogs in Senegal and by Koch (71) in a dog at Dareslam in East Africa.

In South Africa Hutcheon (147) had recognised a disease in dogs related to redwater in cattle and to biliary fever in horses even before the parasite had been demonstrated by Carrington Purvis, and had described a fatal epidemic of this disease which occurred at Herschell, Cape Colony, in the autumn of 1893. Lounsbury says that the disease appears to occur throughout the colony, but to be less prevalent in high inland districts than near the coast, a distribution which coincides with that of the numerical abundance of ticks. In support of the view that the disease is indigenous he cites the experience of old residents, who had recognised the disease at Grahamstown, Mowbray, Humansdorp, Cradock, and other places at dates varying between 1844-54, and quotes an extract from the published letters of Lady Ann Barnard in which the writer refers in 1797 to a disease which attacks all newly arrived dogs. On the other hand he quotes farmers who recollect a time when the disease was apparently first introduced among their dogs. Since the infecting tick *H. leachi* appears to be an African species, it certainly appears probable that the disease was originally endemic in Cape Colony, if not over the whole of Africa.

In India *P. canis* has been recorded by myself (144) in Madras, and by James (149) in Assam. In Madras infection with piroplasma is endemic among native pariah dogs, which may show but few symptoms even when harbouring considerable numbers of the parasite. In adult dogs of this class infection is not common, but a very considerable, though varying, proportion of puppies show parasites. Out of a batch of 18 young dogs brought from different villages in the neighbourhood of the King Institute in October 1905 five were infected,

one showing a heavy infection and two a moderate number of parasites. In August 1906 out of eight dogs one was infected and other batches of which no record was kept showed similar results. The greatest number ever examined without finding parasites was 23 ; parasites were found in the 24th dog.

That the disease occurs in its severest form among European dogs in India is also certain, for, although I have not had an opportunity of seeing it except as a result of experimental infection, several persons ignorant of the nature or significance of the disease have described very exactly to me the symptoms, and a convincing account of an epidemic among the dogs of the Madras hunt a few years ago was given to me by Major Gifford, I.M.S., who, without knowing the cause, noted at the time the extreme bloodlessness of the tissues and the infective nature of the disease.*

It has been suggested by Nuttall that the diseases from the different parts of the world may, especially as they are carried by different species of tick, be due to distinct species of parasites. The answer to such a suggestion will probably require to be based on experiments with regard to immunity, but even considerable variations in symptomatology may arise from differences in age, nature, and condition of the dogs studied by different observers. Thus where the disease is well known as at the Cape and the lives of valuable dogs are at stake its severity is likely to receive special recognition. The effect of the parasite in India is seen under different conditions for should European dogs contract the disease its nature would probably be overlooked, whereas among the pariah dogs one usually sees cases of the disease only in those which have not succumbed in early life.

An interesting feature of the disease in India is that, since the infecting tick of the country is a species found especially in the villages where the indigenous dogs are for the most part congregated, and as in these villages infection is well nigh ubiquitous, it is probable that hunting dogs, if they contracted the disease, would do so from ticks picked up in passing through villages or other haunts of the native dog rather than from those ticks which attach themselves to the dogs in the open country.

In North and South America, Australia, Polynesia, and many other parts of the world the presence of *P. canis* does not appear to have been recorded. How far this is due to want of observation or to the absence of the parasite it is impossible to say, but one is almost forced to conclude from the facts at one's disposal that, as in Texas fever, the disease is prominently brought to view only where susceptible dogs are brought into areas of endemic infection, and that the distribution of *P. canis* is much wider than the record

* *Vide* also Webb (1626).

of its presence would indicate. At any rate its existence may be expected throughout Southern Europe, the whole of Africa, and almost certainly much of Asia.

Symptomatology.

Canine piroplasmosis has been studied in Europe and in South Africa.

The most complete account of the European disease is given by Nocard and Motas (157), who differentiate an acute form ending in death and a chronic form ending most frequently in recovery.

In the acute disease the dog is profoundly affected. Loss of appetite is complete and except in very young dogs, where a high temperature is absent, there is marked fever. In all cases there is intense pallor of the mucous membranes due to blood destruction, and jaundice occurs in about half the cases. Weakness may advance to actual paresis of the hind legs, dogs rising with difficulty and falling if forced to walk. Towards the end the animal becomes comatose.

With the appearance of the first symptoms, even before parasites are present, the urine is albuminous and remains so until death. Hæmoglobinuria was observed in 43 out of 63 cases; it is not constant and when present is often evanescent and may be overlooked. The blood is pale and watery, the corpuscles which normally number 6,000,000 or 7,000,000 per c.m. diminishing with the appearance of the first symptoms and falling at the hæmoglobinuric crisis as low as 2,000,000 per c.m. The hæmoglobin percentage also falls, reaching in some cases $3\frac{1}{2}$ per cent. instead of the normal value of 12—13 per cent. The leucocytes on the contrary, which number in the normal dog from 7,000—8,000 per c.m., increase greatly in number and may reach 40,000 per c.m.

The chronic form is associated with intense anæmia, but rarely with icterus or hæmoglobinuria.

Fever, if it is present, exists only at the outset and lasts only a few days. There is in addition emaciation, anorexia, weakness, and a scurfy skin. Little by little appetite returns and the mucous membranes regain their colour, convalescence taking from six weeks to three months.

The disease as seen in Madras shows nothing in the clinical symptoms to suggest that it is essentially different to the European disease or to the South African disease, the symptoms of which as described by Robertson, Lounsbury, and Nuttall correspond closely with the above.

Young pups may die within 24 hours of the time when parasites first appear in the blood. More usually they live for two or three days. The temperature is generally not raised and there is nothing except the pallor of the tongue and possibly hæmoglobinuria to distinguish the condition from any other rapidly fatal condition in very young pups. In older dogs more characteristic symptoms

are present, but the severity of the disease varies very greatly in different dogs, and even when parasites have been present in large numbers thet symptoms may be so slight as to pass unnoticed. If it is looked for, pallor of the tongue can generally be detected at some stage, but it may not be apparent until after the first few days and may disappear again very quickly after the crisis though parasites may still be present in reduced numbers.

Whilst the two types of the disease described by Nocard and Motas can be distinguished clinically, it is doubtful if they represent essential differences in the type of infection; and a study of reported cases taken in conjunction with my own observations makes it more than probable that infection in all cases goes through certain phases, if the dog lives long enough. Thus in all my cases there has been at the commencement of infection a period lasting for several days during which parasites swarm in the blood, a period which corresponds as a rule with the onset of severe symptoms. If the dog, as often happens, succumbs at the end of, or shortly after this period the type of the disease is that called acute; but should it survive there follows a new phase characterised by the presence of few parasites in the blood, and from this condition the dog may pass to recovery or to death within a longer or shorter period, all gradations between attacks ending at once in recovery and attacks ending fatally after many weeks of sickness being seen.

Thus in the type case 61, given by Nocard and Motas to demonstrate the chronic form, if the dog had died when the temperature fell on the 18th October, all the characters of the acute disease would have been seen. The same initial severity of the symptoms and marked variation of the temperature during the first few days of the illness is seen in case 42, which is given as a type of the chronic disease. In Lounsbury's case No. 17, quoted by Nuttall, there appear to have been two initial attacks of the severe disease and another attack of the same character later on in the disease, a condition which has occurred in my own experience.

The relation of the two types is well exemplified in the following series:— Dogs 135 and 136 were from the same litter and were bitten at the same time by infective ticks. In each the disease developed on parallel lines until, at the period of maximum infection (parasites numbering some 200—300 per field) dog 135 died with hæmoglobinuria (acute type). Dog 136 which also had a maximum infection managed to survive this critical period and died five days later, no hæmoglobinuria being present (sub-acute type(. Dogs 105 and 107, which whilst they were in the same cage were infected by pathogenic ticks also, both showed a severe infection at the start, but dog 105 got over this and, showed the chronic form ending in recovery, whilst dog 107 which died at the time of severe infection showed typically the acute form.

Very young dogs practically never recover from the initial attack so that in them the disease is always of the so-called acute form. Older dogs which do not die from the severity of the infection within the first four or five days appear generally to develop some degree of immunity, parasites after this period rarely being very abundant. It is not unusual for dogs after a long period of low oscillatory temperature and scanty parasites again to have numerous parasites in the blood with high fever, and very possibly they die at this stage. This occurred with dogs 95 and 96. These were dogs from the same litter inoculated on the 24th of October 1906 with blood of a not very virulent strain and they developed a mild attack in which parasites averaged about two per field and the temperature rose to 103°. At various periods after this they were observed to have a few parasites in their blood. On the 21st November 1906 the dogs were observed to be very ill and blood examination showed abundant piroplasmata. On the 22nd November 1906, dog 19 died and dog 20 died the next day, both having very greatly enlarged spleens. It is quite possible that such cases are due to reinfection by ticks, but exacerbations of parasites have been seen in dogs in which fresh tick infection was impossible.

Dogs which get over the initial invasion may die with progressive anæmia and emaciation, or they may recover. As noted by Robertson many dogs which appear to have recovered succumb eventually. In some cases this may possibly be due to reinfection by ticks infected by the dog itself, and is very characteristic of the disease as seen in dogs at liberty. Very mild infections, in which the dog exhibits no sign of illness, and recovery is rapid and complete, are also quite common even among European dogs.

Temperature.—All observers agree that the first sign of infection is a rise in the temperature. This may occur before the dogs show any sign of ill health, but it has been associated in all my cases that have been examined at this time with abundant parasites in the blood. Twenty-four hours later the dogs usually show obvious signs of illness, though as yet no great pallor is visible. Pallor, however, becomes apparent a little later and then the tongue may in severe cases be the colour of pipe clay. A change of expression in the eyes is also noticeable, possibly a result of the increased whiteness of the sclerotic, though the eyeball also appears unduly prominent.

Destruction and re-formation of red cells.—The rapidity with which the red cells are destroyed is the most characteristic feature of the disease, and can be followed even by the unaided eye in the rapidly increasing thinness of the blood. In the loss of a million or more corpuscles in a few hours it almost exactly resembles blackwater fever in man, a disease in which the destruction of red corpuscles is every bit as rapid and as extensive. The rapidity with which new corpuscles are made is also very great and is well exemplified in case No. 61

of Nocard and Motas where the number rose from 2,430,000 to 4,340,000 in five days and in another two days reached 5,100,000.

Hæmoglobinuria.—Hæmoglobinuria is described as frequent in both the European and South African disease. It is also present in the Madras disease, though I have never seen the urine of the dark porter colour so characteristic of severe cases of blackwater fever in the human subject. Nocard and Motas describe the urine as containing hæmoglobin in three out of six naturally infected cases and in 43 out of 63 dogs suffering from the inoculated disease. They note that it is sometimes very fugitive and may be overlooked. Nuttall notes hæmoglobinuria in all his cases except one, but describes the urine only as dark iodine colour. In the cases seen by me the urine was most frequently of a light reddish tint or of a darker prune juice colour. Very often when hæmoglobinuria is not present the urine is pale; at other times it is of a dark yellow. I have seen hæmoglobinuria only in dogs which have died during the initial severe attack. In dog 135, which died at a period of very intense infection, hæmoglobinuria was present, but in dog 136, which had passed through nearly as severe an attack and died five days later, the urine in the bladder did not contain hæmoglobin. Even dogs which die of the acute disease do not necessarily show distinct hæmoglobinuria.

Nervous symptoms.—In very severe cases there is nearly always marked paresis amounting to distinct paralysis in the hind legs. This is usually observed in dogs which die at the first invasion.

Immunity.—The only observers who have followed closely the process of immunity in canine piroplasmosis are Nocard and Motas. These authors demonstrate not only a high degree of immunity resulting from previous infection lasting many months, but also show that the blood of immunised dogs has a marked bactericidal effect upon the parasites, mixture *in vitro* with four or five times its volume of serum from an immunised dog rendering virulent blood ineffective.

The serum of immunised dogs has, however, but slight effect in preventing or modifying an attack.

Pathological Changes in Canine Piroplasmosis.

The pathological changes which occur as a result of infection by the different piroplasmata are in the main similar; the changes in East Coast fever, however, are peculiar to this disease. In all cases the chief characters noticeable at the autopsy are the extremely watery condition of the blood and the pallor of the tissues. The degree of icterus varies in the different diseases; it occurs only

TABLE 1.—*Showing alteration in weight of the viscera in acute and chronic cases.*

Number of dog.	Weight of dog in grammes.	Weight of spleen in grammes.	Percentage of body weight.	Weight of liver in grammes.	Percentage of body weight.	Weight of both kidneys in grammes.	Percentage of body weight.	Date parasites first appeared.	Date of death.	Duration of disease in days.	Remarks.
106	898	2·9	·323	38·6	4·4	12·9	1·43	.....	20th Nov. 1906	...	Normal dog.
118	658	1·3	·197	34	5·16	9·3	1·41	.....	21st „ „	...	Ditto.
107	981	6·1	·621	48	4·77	21·7	2·21	16th Nov. 1906	20th „ „	4	Acute. Hæmoglobinuria at death.
121	825	13·8	1·69	55	6·66	16	1·94	17th „ „	21st „ „	4	Acute. Urine dark brown. Trace of H.
122	807	10·3	1·26	65·6	8·12	12·5	1·54	28th „ „	1st Dec. „	4	Acute. Paralysis of hind legs. Hæmoglobinuria.
133	360	1·4	·39	13·5	3·75	4·8	1·33	29th „ „	3rd „ „	5	
147	420	1·5	·357	14·9	3·55	4·7	1·15	15th Dec. „	18th „ „	3	Dog killed. Probably would have died.
148	454	·9	·2	17·7	3·9	4·3	·947	15th „ „	18th „ „	3	Ditto ditto.
149	486	1·2	·247	21·4	4·40	5·8	1·19	15th „ „	18th „ „	3	Ditto ditto.
135	854	5·5	·644	55·7	6·52	13	1·52	18th „ „	19th „ „	1	Intense infection. About 300 parasites per field. Hæmoglobinuria.
136	985	9·5	·9	67	6·80	23	2·33	18th „ „	23rd „ „	5	Urine pale.
139	1,900	28	1·5	125	6·58	21	1·10	18th „ „	29th „ „	11	Dog killed. Probably would have recovered.
146	757	19	2·51	52·8	6·97	16·3	2·17	1st Jan. 1907	4th Jan. 1907	3	Dog killed. Probably would have died.
144	925	12·5	1·35	73	7·9	12·5	1·35	.....	10th „ „	...	Urine deep yellow.
116	...	46·6	...	142	...	25·7	...	30th Oct. 1906	22nd Nov. 1906	23	Chronic disease.
117	1,459	46·7	3·2	115·7	7·93	18·8	1·28	30th „ „	23rd „ „	24	Ditto.
131	2,210	27·7	1·25	211·7	9·57	20·6	·932	.....	10th Jan. 1907	...	Ditto.
132	1,600	18·5	1·15	95·7	5·90	23·9	1·49	.....	10th „ „	...	Ditto.
140	2,559	25	·977	210	8·20	24	9·37	.....	.....	...	Ditto.

in a proportion of cases of Texas fever and malignant jaundice of the dog, but is especially noted in infection with *P. equi*. Hæmorrhages are seen in Texas fever and in biliary fever of the horse, as well as in East Coast fever, but they are not common in malignant jaundice of the dog. Oedema under the jaw occurs in East Coast fever ; and œdema of the subcutaneous tissues especially over the belly is described by Smith and Kilborne in Texas fever. In the dog I have sometimes seen rather a characteristic œdema over the sacrum, and in one or two acute cases this was associated with a gelatinous exudation about the lower end of the spinal canal. The presence of abdominal and pericardial fluid in canine piroplasmosis has been described by several authors, and the former I have found especially common.

Enlargement of the spleen is a common result of piroplasmosis and is very marked both in Texas fever and in biliary fever of the horse. In the dog enlargement of the spleen to three or four times its normal size is described by Nocard and Motas, who note that the gross changes in the organs are more marked the longer the disease has lasted. Nuttall and Graham Smith state that enlargement of the spleen and other gross lesions were not noticeable in their cases, which were all of the acute type.

Even in very acute attacks I have generally observed some enlargement of the spleen, which is more tumid than the normal organ, and is when first exposed of a dark purple colour. In severe cases which have lasted some time, the enlargement may be very considerable, as will be seen from the table giving the weights of the organs in a number of cases. When greatly enlarged the organ is firmer than normal and retains its shape when removed from the body. In consistency it resembles the spleen of kala azar.

Changes in the liver are no less profound than those in the spleen. In Texas fever Smith and Kilborne note enlargement, congestion, bile injection, and fatty degeneration. Congestion and dilatation of the bile capillaries are noted by Bowhill in horses, and by Dale in donkeys, which have succumbed to piroplasmosis. In East Coast fever infarcts occur in the liver and other organs. In the dog Nocard and Motas describe engorgement and an appearance resembling the liver of cardiac disease. In my own cases the liver has always been pale in colour and in long standing cases shows mottling, probably due in part to fatty changes. In all chronic cases there is an increase in weight as shown in the table.

The condition of the kidneys in my cases has varied. In many acute cases they have been normal in appearance, but in others, especially when hæmoglobinuria was present, they have been enlarged and congested. In chronic cases they are often enlarged and pale in colour, their consistence being very firm. In other cases, except for pallor, they are not abnormal.

The other organs show appearances attributable to intense anæmia, rarely any other change. But the red marrow is increased in amount and in chronic cases has a fætal character.

Morphology of the Parasite.

With the exception of the minute parasites of East Coast fever all the known piroplasmata are somewhat similar in appearance and approximately pear-shaped forms occur. Nocard and Motas state that *Piroplasma canis* is morphologically indistinguishable from *Piroplasma bigeminum*, but Robertson describes *Piroplasma canis* as more oat-shaped, an opinion in which Nuttall supports him. In the Madras parasites the difference is quite distinct, *Piroplasma bigeminum* being much more strictly pear-shaped than *Piroplasma canis*, whilst it is at the same time smaller. Measurements of the same species of piroplasma by different observers often vary considerably, a result one might expect, since both apparent and real variation in size occur under different conditions of preparation and of infection. For *Piroplasma canis* Piana and Galli Valerio give 2·5—3·5 μ, Marchoux 2—4 μ, Nocard and Motas note large forms occupying half the red cell, Nuttall and Graham Smith give the measurement of the African parasite as from ·7 to 1·2 μ though they note that larger types reach 3·6 μ and occasionally even 4·5 to 5 μ. In the case of *Piroplasma bigeminum* Smith and Kilborne in America give ·5 μ, Laveran and Nicolle in Constantinople 1—3·5 μ, and Ziemann in Venezuela ·75 to 3 μ so that smaller forms of this parasite appear to be more frequent. For *P. ovis* Bonome gives 1—3 μ and Laveran and Nicolle 1—2 μ. *P. equi* is considerably smaller, the usual size being given by Laveran as from 1—1½ μ the largest forms not exceeding 2½ μ.

In all species multiple infection of the red cell is seen, the characteristic twin parasites being characteristic of all except *P. parvum*. In *P. equi* forms arranged in the manner of a cross are stated to be characteristic but similar forms occur in *P. canis* and are especially noticeable in blood taken *post-mortem* from the heart. *P. equi* is figured by Laveran(169)as small round forms, but in the figures given by Bowhill (163*a* and *b*) this parasite does not differ very greatly in general appearance from *Piroplasma canis*.

In *Piroplasma canis* all observers have noted the infrequency with which corpuscles contain an odd number of parasites. Graham Smith, dealing with large numbers, has demonstrated this clearly and gives instructive figures regarding the number of parasites in red cells. These will be referred to again in another connection. According to this author single forms predominate, forming from 50 per cent. to 76 per cent. of the total forms in the peripheral blood and from 29 per cent. to 50 per cent. of those in the organs. Next in frequency come the double forms which as a rule form only from 20—30 per cent. of the whole,

though even in the peripheral blood they are noted as rising to 43 per cent. In the organs they averaged about 40 per cent. and reached in the case of the marrow 52 per cent. of total forms. In striking contrast to these figures are those showing the percentage of infected cells containing more than two forms. In the peripheral circulation cells infected with four parasites number only from 2—5 per cent., those with eight forms less than ·05, and those with 16 forms from ·004 to ·01 of the whole. The figures for cells in the viscera containing more than two parasites are higher but the numbers are still far below those of the single and double forms, the highest percentage given for cells infected with four parasites being 12·39 and for those containing 16 forms ·01. Corpuscles containing an odd number of forms are comparatively very few, corpuscles in which there are three parasites numbering only ·7 of the whole, whilst those containing higher odd numbers are still rarer.

My own counts, though they have not been based on such large numbers, correspond in the main with those given by Graham Smith, and they also show that, no matter at what stage of infection the blood be taken, the forms maintain an approximately similar relationship to one another.

Thus corpuscles containing single forms have varied from 22 per cent. to 81 per cent., but are usually 50—60 per cent.; those with double forms from 18 per cent. to 50 per cent. but are usually 30—40 per cent.; and those with over four forms rarely exceed 20 per cent. and are usually from 5 to 10 per cent.

As will be seen later this general order of frequency, although it is never completely upset, is subject to certain definite and important variations which have a cyclical significance.

The piroplasmata are described by the earlier observers as parasites of simple form possessing a single nuclear mass of chromatin; and except in *Piroplasma canis* little further structure has yet been demonstrated. Koch, however, mentions in *Piroplasma bigeminum* a large and a small mass, the latter of which is situated towards the thin end of the parasite; and Fantham notes in *P. muris* nuclear characters of some complexity.

Piroplasma canis has been the subject of more detailed study and a large and somewhat perplexing number of forms have been described. A very complete study of the parasite in the living state has also been made by Nuttall and Graham Smith (160). To facilitate description it will be advisable to mention the various forms noted by different observers, although most, if not all, are but stages in growth and fission.

Amœboid forms.—All observers who have studied the living blood note the protrusion of pseudopodia and movements so active as sometimes to move the corpuscle in which the parasite is situated. Nocard and Motas state that parasites exhibiting amœboid movement are best seen during the febrile state, and that

after this they lose their amœboid properties and remain immobile in the centre of the cell.

Nuttall and Graham Smith distinguish between two forms of amœboid parasite, those which show only slight amœboid movement and those with well marked pseudopodia.

Both forms can be correlated with forms seen in stained preparations. The actively amœboid forms appear in stained films as triangular, elongate or irregular forms, and when parasites of this type are watched in the living state they will be seen constantly to assume a shape more or less triangular as shown in Pl. 1, fig. 12. Another very common appearance is that of a long almost vermicular form stretched across the corpuscle (Pl. 1, figs. 13 and 16). Ordinary amœboid shapes are also seen in stained films and usually represent parasites in process of changing from one of the above-mentioned shapes. At certain times, notably when parasites are extremely abundant, appearances which suggest extraordinarily active amœboid movements are seen, and parasites exhibiting these movements may show in addition to pseudopodia the long flagella-like processes described by several observers.

The triangular, elongate, and irregular forms represent undoubtedly the actively growing parasite which in the living state is in active amœboid movement.

Pear-shaped forms.—These are so characteristic and well known that a description is scarcely necessary. The majority of the parasites come under this heading, and as a rule when more than a single parasite is present in a cell each is more or less pear-shaped. When four, eight or more parasites are included in a single cell they are nearly always more or less pear-shaped, but with a tendency to become somewhat polygonal. Of pear-shaped parasites several distinct varieties are seen. The most typical are those resulting from fission, but a pear-shaped form may be assumed at the end of the period of growth and prior to division. These are large parasites readily distinguishable from the smaller and more strictly pear-shaped forms which are seen as a rule most typically in cells containing four or eight parasites. Pear-shaped forms also vary much in size and in the reaction of the protoplasm to stains. Those staining a more or less uniform and comparatively dark blue colour are the most common. In thin portions of films these are less pear-shaped than in the thicker portions where the parasites are contracted laterally.

The pear-shaped forms resulting from the fission of a single large form are very often rather globular bodies and most frequently stain more lightly than the forms just mentioned; the chromatin also is usually situated more peripherally than it is in the multiple forms where the chromatin is usually nearly central in position.

The formation of the pear-shaped forms by fission occurs in two or even three distinct ways.

1. In the case of certain large parasites, characteristic of early invasion and of infection in young dogs, the division takes place in a manner described by Nocard and Motas. After the chromatin has divided a dark line forms across the parasite in the centre of which a cleft is later seen. This gives rise to two closely approximated bodies. (Pl. 1, figs. 4—7.)

2. In parasites which immediately before division are round or oval in shape, division takes place so as to give rise to the appearances first described by Nuttall and Graham Smith who believe this to be the usual method. Signs of division are first evidenced by the two masses of chromatin surrounded by more or less protoplasm passing outwards from the parasite. In some cases the chromatin bodies have so little protoplasm that they appear to be extruded. In others about a third of the protoplasm surrounds each mass giving rise to the trefoil appearance noted by Nuttall and Graham Smith. Eventually in all cases the whole of the protoplasm is drawn away from the original body of the parasite to one or other of the chromatin masses, which become the nuclei of two new parasites joined at first by their apices, but afterwards free. (Pl. 1, figs. 21—23 and figs. 1, 1—6, page 21.)

The various stages and varieties of this process are responsible for many of the appearances seen in stained films.

3. A method of division, almost if not quite as common, takes place when the parasite is stretched across the cell. The long oval becomes constricted in the centre and the two ends passing away from one another become two pear-shaped bodies. (Pl. 1, figs. 9—14 and figs. 1, 1—6, page 21.)

4. It happens not infrequently that a second fission is in progress even before the first is completed so that four forms are produced almost simultaneously.*

Early infection forms.—Nocard and Motas describe at the commencement of infection large forms nearly filling the corpuscle. They note that these are specially large in young dogs. In my cases the first parasites seen have generally been of this character and they must be looked upon as a distinct type, though the transition of these forms into the pear-shaped forms is easy to follow. A few parasites of this type are generally seen at the commencement of infection and within a very short time they become comparatively abundant in the blood. In young dogs, as noted by Nocard and Motas, the single forms are especially large and may reach 4 μ in diameter occupying nearly all the corpuscle.

The chromatin mass is usually peripherally situated. The protoplasm is reticular and slightly refractile. In the early stages ring forms somewhat

* This gives rise to the appearance of budding described by Kinoshito but it is very doubtful whether a permanent "reliquat" is left.

resembling those of the malarial parasites are common. These have to be distinguished from the apparent ring forms with refractile centre described later. As the parasite grows the ring tends to disappear and changes of an amœboid nature take place, the parasite becoming racket-shaped, triangular, oval, or elongate. Eventually a large slightly oval body is formed in which the chromatin has become separated into two. Fission of these forms usually takes place in the manner described by Nocard and Motas rather than in that described by Nuttall and Graham Smith. By development into four forms the parasites approximate either to the ordinary pear forms, by the chromatin becoming more central and the protoplasm bluer, or into the round refractile type, the chromatin in this case being pushed still more to the periphery.

Round refractile type.—Nocard and Motas make special mention of these forms which are referred to only incidentally if at all by other observers. At times almost all the parasites are of this type ; at others they are found lying side by side with ordinary pear-shaped forms so that the appearance is not one due merely to fixation or staining. These parasites are often round, but where they form members of a group they are usually slightly pear-shaped. They have a narrow darkly staining portion and a brightly refractile centre. The chromatin is not only peripherally situated but is flattened. Nocard and Motas describe it as sometimes occupying nearly two-fifths of the periphery, and such an extended area is quite common. Between these and the large early infection type there are transitional forms which as they divide to form groups of two and four may take on the characters noted. Groups of four are the commonest and these show the characters very markedly, but groups of eight may occur.

In the fresh condition these forms are quite motionless, but in stained specimens amœboid processes of the kind shown in Pl. I fig. 27 are occasionally seen.

Similar forms are seen in the large bovine piroplasma of calves of Madras and are also figured by Myajima and Shibayama (102) in the piroplasma of cattle in Japan.

The significance of the forms is not clear.* Nocard and Motas consider them a resting stage and they note their presence after the febrile period, an observation which I can confirm. They can scarcely, however, be due merely to immunity following an attack, for they may occur along with the ordinary type of parasite.

Ring forms.—These have been especially noted by Luhe. True ring forms occur, especially in the early invasion forms, but also in the case of other single parasites. In some cases one or more true vacuoles are also present.

* Kinoshito describes certain forms as gametes, but the evidence given in favour of this view is not very convincing.

Vacuolated forms.—Large oval or irregular bodies shewing vacuoles are not uncommon at the stage of active growth.

Flagella-like processes.—These have been described by Pound, Bowhill, and Le Doux in *Piroplasma canis* and by Lignières and Bowhill in the large piroplasmata of cattle. They have been described by Nuttall and Graham Smith both in free parasites and in intracorpuscular forms. In the former case a flagellum-like process often projects from the thin end of the parasite. In stained films this is only occasionally seen, but in parasites examined in the fresh state it is not only very frequent, but is seen to exhibit active movements.

These authors describe in intracorpuscular parasites the occurrence of one or more very long and thin wavy flagella ending in a minute knob or more rarely in a fine point.

These fine pseudopodia are frequently seen in the actively growing forms at the height of invasion and, as noted by Nuttall and Graham Smith, they are a feature of cultures. That they have any sexual function as supposed by Doflein is improbable and they appear to represent merely an exaggeration of amœboid movement associated with specially active absorption of material by the parasite.

Post-mortem forms.—Forms seen in the blood taken from the heart a short time after death are as a rule reduced in size and globular. They also stain intensely, having an appearance quite different from that of parasites prepared in the ordinary way from living peripheral blood. Not infrequently tags of protoplasm are present and sometimes these may be quite detached from the parasite. The chromatin, though obscured by the dark staining protoplasm, still stains and is central in position. Unchanged forms may also be present. (Pl. 1, figs. 41 and 48.)

Free forms.—Free forms are described by nearly all observers, and they are a conspicuous feature of films taken at certain times. They may be single, but are more frequently seen in groups of two, four, eight, or sixteen and have evidently arisen by rupture of a cell a short time before. In many cases the outline of an enlarged and colourless corpuscle is visible. Free forms differ from the included ones in that they stain a much deeper blue and are almost invariably pear-shaped or show an approximation to this shape, though a somewhat angular outline is characteristic. The size of free bodies varies considerably, and very large forms are often seen as the result of rupture of a cell containing two parasites only. When one of a group of sixteen they are usually much smaller.

The chromatin of the free forms is nearly always more or less centrally situated.

Nuttall and Graham Smith consider that in normal development included forms, after division into two, are liberated and again penetrate cells, but it is

evident that many parasites go on to the formation of four, eight, or more forms before they are liberated by rupture of the cell, a condition which, as we shall see later, probably represents the commonest cycle of development. The matter has some importance since it is quite possible that not all parasites on being liberated by rupture of the cell are in a fit condition to penetrate fresh cells. Thus we may distinguish, for example, between forms liberated immediately prior to division, those liberated after a single process of fission, and those resulting from fission repeated many times. Further observations on these points are required.

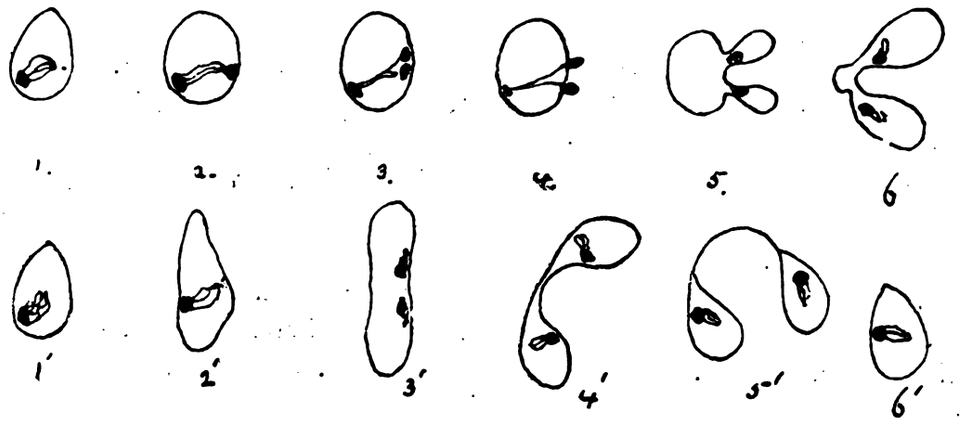

FIG. 1.—Showing movements of Chromatin in the process of binary fission.

Fig. 1.—Parasite showing early signs of fission. Appearance of blepharoplast.
Fig. 2.—Passage of chromatin to neighbourhood of blepharoplast.
Fig. 3.—Division of new chromatin mass into two.
Fig. 4.—Protrusion of the two new masses and passing over of remainder of the original mass.
Fig. 5.—Protoplasm flowing past the two new chromatin masses.
Fig. 6.—Showing apical position of the chromatin masses due to protoplasm flowing past them.
Fig. 1'.—Parasite prior to changes leading to fission.
Fig. 2'.—Similar stage to 1, but parasite elongated.
Fig. 3'.—Similar stage to 5. Parasite stretched across corpuscle.
Figs. 4', 5', 6'.—Final stages in second type of division.

The chromatin.—A large compact mass of nuclear nature has been described by all observers. Later Schaudinn(10)and Luhe(7)have drawn attention to the presence of a minute punctiform mass, which they consider to be of the nature of a blepharoplast. Nuttall and Graham Smith note that in many of the parasites three distinct masses of chromatin are found, a large and compact mass, the nucleus, a minute dense punctiform mass usually lying in the immediate neighbourhood of the nucleus, the blepharoplast of Schaudinn and Luhe, and a third lightly staining irregular loosely packed or reticulated mass hitherto undescribed. These authors further describe and figure a great many variations in the

position of these masses in different forms of the parasite, and also refer to other appearances which I shall discuss later. Fantham* has also observed the loose area noted by Nuttall and figures a relation of the blepharoplast to this such as is shown in fig. 1, No. 1. To demonstrate the chromatin I have used various degrees of colouration of Giemsa's stain and the very intense modification of Romanowski's stain described by Wright, in which by careful washing of intensely stained specimens with weak alcohol very clear results can be obtained.

Variations in the position of the large chromatin mass appear to be due mainly to the positions which it has occupied at various times after fission.

It lies as a rule somewhat near the periphery so that it may appear from different points of view on either side or in the centre of the parasite. Again, as after fission it passes round the periphery, it may be at various levels between the base and the apex of the pear.

In the multiple forms it is more central in position. In the refractile forms it is pressed to the periphery and may be flattened or drawn out to a rod shape.

The blepharoplast is often seen as a distinct and very markedly punctiform body. It is, however, by no means always present. In my specimens it has generally been associated with changes in the third area of chromatin and with division of the parasite. (Pl. 1, figs. 32—39 and fig. 1, page 21.)

The loosely packed area described by Nuttall and Graham Smith is almost invariably present, and its apparent absence is generally a result of imperfect fixation or staining; in certain small round forms of infrequent appearance it is, however, often not visible.

Nuttall and Graham Smith figure this mass as in some cases continuous with, and in others separate from, the main mass. As a result of close observation I am convinced that it is rarely, if ever, really separate from the main chromatin mass, though its main bulk may be so; as a rule it is obviously continuous with and appears as an extension of the main mass.

In many preparations the area consists of a fine irregular reticulum of chromatin threads, in others it appears as an area of homogeneous material having clearly defined edges from which there may arise one or more fine chromatin filaments. In some cases the extension clearly consists of two or three long chromatin filaments, which can be traced almost the full length of the parasite.

In some cases the filaments are aggregated to form a thick cord, in others they are more spread out. In the large "early invasion" forms a fan-like arrangement is common. In the round refractile forms the main mass is flattened upon the periphery with a long straggling projection of loose chromatin usually lying

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* Private communication.

along the periphery, but sometimes spread as a thin streak over the refractile centre (Pl. 1, fig. 29).

An arrangement of the chromatin of the same nature is seen in the developmental forms of piroplasma in the tick. In both situations this extended or filamentous portion of the chromatin first heralds division of the parasite. In single forms in the blood which are in the early stage of growth the chromatin consists of the main mass with a small extended area (fig. 1, No. 1). As growth proceeds and division approaches, the extended area becomes more pronounced and spreads further from the main mass. It eventually points as it were at some portion of the periphery of the parasite, and very frequently a condensation of its substance at this point is seen (fig. 1, No. 1). It is at this period that the blepharoplast is most constantly seen and it very frequently bears a relation shown in fig. 1, No. 1, an arrangement which Mr. Fantham tells me he has also observed. As fission approaches, the chromatin from the main mass appears to pass over and accumulates in the position originally occupied by the blepharoplast, thus giving rise to the appearance of two chromatin masses connected by a bridge of chromatin (fig. 1, No. 2). In some cases fission would then seem to take place by the two masses separating and becoming the nuclei of the new parasites. In many cases and possibly in all a more complicated process takes place*.

After a certain amount of chromatin has accumulated in the new position it divides into two portions which pass out of the parasite as previously described (fig. 1, Nos. 4 and 5). At this stage a portion of the original mass is often still present, the parasite then showing three chromatin masses joined by delicate threads, the representatives of the extended area (fig. 1, No. 4). Later, the original chromatin all passes over to one or other of the new masses, sometimes leaving a minute mass which may simulate a blepharoplast (fig. 1, No. 4). A somewhat similar process can also often be detected in the elongated forms of parasite which divide by a constriction of the centre. In this case the most common arrangement of the chromatin is for the original mass to be upon one side about the middle of the parasite, and for the blepharoplast, and later the new chromatin masses, to be upon the other side, also about the middle (fig. 1, No. 21). Being so close together the relation of the masses is not so easy to follow as in the large oval or round forms. In a common method of division the blepharoplast arranges itself close alongside the original mass (Pl. 1, fig. 36). But here the proximity of the chromatin masses usually prevents the nature of the changes from being followed.

* Since this paper has gone to press Nuttall and Graham Smith have published a very similar account of these changes. They describe, however, fission of the original mass into two, one of which masses again divides. The passing over of chromatin and the appearance of the blepharoplast at a certain stage are not described.

In the multiple forms arrangements for a second fission are usually we advanced very shortly after the preceding division, and no sooner has the extended area divided into two then, arranging itself transversely to the length of the parasite, it again " points " and becomes the channel for the separation of the chromatin (fig. 1, No. 61).

Free forms may show any stage of the above, but most commonly have a nucleus situated near the pointed end of the parasite with an extended area and blepharoplast arranged as in the preliminary stages of preparation for division. In some cases the extended area is apparently at rest and forms a more or less irregular body lying to one side of the nucleus. In the early invasion forms division appears to take place after direct division of the large chromatin mass.

Cultivation forms.—Attempts to observe development of piroplasma *in vitro* have been made by several observers. Lignières observed what he considered a multiplication of the parasite, but others have thought this appearance to be merely the result of the dissolution of corpuscles leading only to an apparent increase in parasites. Lignières has also figured two parasites in a corpuscle from which projected long and fine flagella, an observation believed by Doflein to show that flagellation occurs in piroplasma as it does in the malarial parasite. It is more probable that the flagella figured represented only processes of the red cell, such as are frequently seen in fresh films under certain conditions. Nocard and Motas kept defibrinated blood rich in parasites at 37°C. They never saw any multiplication, but observed that the parasites became smaller and rounder, due to a kind of condensation, and that the nucleus retained its staining properties for a long period, suggesting that the parasites were still living ; experiments with blood kept fluid in the gut of the leech were equally unsuccessful in showing any development. These authors note that a very intense phagocytosis is sometimes seen *in vitro.*

Nuttall and Smith have made observations on the changes in the blood from the heart taken immediately after death and kept at room temperature in the dark. After 24 hours they found the majority of the parasites free in the serum, whilst others were in the leucocytes. At the end of 48 hours intracorpuscular parasites showed very little change in their appearance, but many of them had very well-marked processes and flagella. Sometimes the parasite occupied a clear space in the corpuscle. Free forms were numerous and occurred in groups of 30 or more. The parasites comprising these groups were much changed being irregular or rounded in shape and varying from minute organisms one-sixth the diameter of a corpuscle to parasites of a size nearly equal to that of corpuscle; but no definite development was observed.

In marked contrast to these results are those of Kleine (187), who following

Koch has obtained positive results *in vitro* with *Piroplasma canis*. Kleine used the blood of young pups which had been injected with infective blood. A certain time after inoculation parasites were found to be very abundant. The dog was then killed and the blood taken was mixed under sterile precautions with an equal quantity of normal saline and kept at 27° or room temperature. Under these conditions the parasites underwent development sending out long processes as in the development described by Koch as occurring in the tick.

There are possibly several points in favour of cultural results not, so far as I know, recognised by others who have made cultural experiments.

1. The marked bactericidal effects of the blood noted by Nocard and Motas is probably minimised by the early date at which the blood is withdrawn, when puppies are killed so soon as numerous parasites appear.

2. To judge by the susceptibility of very young pups their blood would appear to be in the most suitable condition for culture.

3. The period of the attack during which the blood is taken has considerable importance and in my own experiments disappointing results were sometimes due to the blood having been taken at a time of crisis when an intense phagocytosis removed practically all the parasites present.

In many of my cultures, though changes occurred in the parasites, it is doubtful whether any development occurred. Most usually at the end of twenty-four hours parasites were seen to have become stellate in form (Pl. 1, figs. 45 and 46), sometimes lying in a vacuole in the red cell and sometimes free, but at the end of forty-eight hours no further changes took place.

In other cultures, especially those taken early in an attack, different conditions prevailed. Forms included in the red cells usually showed at the end of twenty-four hours either an extraordinary development of flagella-like processes (Pl. 1, figs. 43 and 44) or remained unaltered. A proportion of the parasites, especially the free forms, on the other hand exhibited appearances resembling those of the early changes seen in the tick. They were increased in size and were often angular or showed projections, but never the long spikes described by Kleine (Pl. 1, fig. 47).

Progress of Infection.

Infection in the case of *Piroplasma canis* results from both intravenous and subcutaneous inoculation of infective blood. The period of incubation as first observed by Spreul and Purvis is shorter in the former case. Nocard and Motas note the onset of fever three to five days after intravenous and five to six days after subcutaneous inoculation, but parasites have been seen by these observers so soon as 36 hours after intravenous inoculation, and in a case of

Nuttall and Graham Smith symptoms appeared only after 10 days. In the case of subcutaneous inoculation Robertson notes the occurrence of symptoms in three, four and five days, and Lounsbury six and seven days in two cases.

In my own cases parasites have usually been seen on the 4th or 5th day after subcutaneous inoculation, and have become numerous the day following their first appearance, when symptoms are first noticeable. Nocard and Motas and others have used inoculation as a means of studying the disease, and when young dogs are used the certainty of infection is as great as that usually associated with bacterial infections. An interesting point has been raised by Robertson who, after carrying the disease by inoculation through thirteen dogs, considered the virulence of the parasite to be enhanced; my own experiments are more in favour of an opposite conclusion, and it was noticeable that infection by ticks gave rise to much more severe types of the disease than did inoculation. The following series among batches of young dogs of the same litter inoculated in the course of cultural experiments shows the susceptibility of dogs to artificial infection and the length of the incubation period under these conditions.

On 24th August 1906 an emaciated dog showing a fair number of piroplasmata was brought to the laboratory. On the same day it was chloroformed and about half a cubic centimetre of heart blood inoculated subcutaneously into four young puppies numbered 44, 45, 46 and 47. On the 30th August 1906 dog 44 was seen to be about to die and its blood was found swarming with piroplasma. At the same time dog 45 had a few large round forms, dogs 46 and 47 being negative. Dog 44 died, and on the 31st August 1906 all the others showed parasites and were chloroformed on this or subsequent dates.

On the 25th August 1906 a dog showing piroplasmata in the blood, but not obviously ill, was brought to the laboratory and on the same day four young puppies numbered 48, 49, 50 and 51 were inoculated subcutaneously with about half a cubic centimetre of blood. Of these dogs Nos. 50 and 51 were from an older litter than 48 and 49. On the 31st August 1906 dogs 48 and 49 both showed abundant large forms and the blood of dog 50 was swarming with parasites. Dog 51 showed a few forms only, but next day its blood contained abundant parasites. On the 31st August 1906 two half-grown dogs, Nos. 52 and 53, were inoculated with 1 c.c. of blood from dog No. 45. Dog 52 died on 7th September 1906 and dog 53 was very ill and its bood contained numerous piroplasmata.

On the same date (August the 31st) two half-grown dogs, Nos. 55 and 56, were inoculated with $\frac{1}{4}$ c.c. and 3 c.c. respectively of blood from dog 50. On the 4th September 1906 dog 55 showed scanty and dog 56 numerous parasites. The next day both showed numerous parasites.

TABLE II.—*Showing time at which parasites first appeared in the peripheral circulation after the bite of infected ticks.*

Number of dog.	Bitten by	Date bitten.	Piroplasma first seen.	Period of incubation in days.	REMARKS.
105	Nymphs . .	13th October 1906 .	17th October 1906 .	4	
107	Nymphs . .	13th October 1906 .	17th October 1906 .	4	
109	Nymphs . .	4th November 1906.	12th November 1906	8	
110	Nymphs . .	4th November 1906.	12th November 1906	8	
111	Nymphs . .	4th November 1906.	8th November 1906.	4	
112	Nymphs . .	4th November 1906.	8th November 1906.	4	
121	Nymphs . .	13th November 1906	17th November 1906	4	Negative, 16th November 1906.
122	Adults . .	22nd November 1906	28th November 1906	6	
127	Adults . . .	29th November 1906	4th December 1906 .	5	
129	Adults . . .	29th November 1906	4th December 1906 .	5	
131	Adults . . .	11th December 1906	21st December 1906	10	First infection probably earlier as not examined previously.
132	Adults . . .	11th December 1906	21st December 1906	10	Ditto ditto.
135	Adults . . .	10th December 1906	18th December 1906	8	Swarming piroplasmata when first examined December 1906. but free on 15th.
136	Adults . . .	10th December 1906	18th December 1906	8	Ditto ditto.
139	Adults . . .	10th December 1906	18th December 1906	8	Ditto ditto.
140	Adults . . .	10th December 1906	18th December 1906	8	Ditto ditto.

On the afternoon of 22nd October 1906 half-grown dogs Nos. 98 and 99 were inoculated in each case with ½ c.c. of infective blood. On the morning of the 26th October 1906 scanty piroplasmata were found in dog 98, but none in dog 99. Next morning both dogs showed infection.

Under conditions of natural infection by ticks the incubation period has by most observers been placed at from 10 to 15 days. Lounsbury in his long series of feeding experiments with infected *H. leachi* has usually observed incubation periods of from 10 to 12 days, but in some cases the period was extended to 14 and even 21 days. In Nuttall and Graham Smith's cases the incubation periods in the cases of three dogs infected by ticks were 13, 15 and 16 days respectively.

The periods differ very markedly from those in my own experiments in which infection was brought about by means of numerous recently and heavily infected *R. sanguineus*. As will be seen from the table the time for the appearance of parasites in some cases has been so short as four days. In one or two instances a much longer incubation period was noted, but these cases occurred before rigid precautions had been taken to exclude chance infection and cannot be relied upon as can those which occurred in the course of experiments given in full later on, and in which the possibility of error was eliminated by precautions to be described. That dog after dog succumbed to infection at or about the same time after exposure to the bite of infected ticks whilst controls remained well throughout can scarcely be interpreted in any other way than that, under the conditions then present, the naturally produced disease followed extremely quickly upon the bite of the infecting ticks. The following list of infections acquired naturally in the laboratory also points to an extremely short incubation period in the disease under certain circumstances. The cases occurred among young healthy pups brought to the laboratory and allowed to run about at a time when the laboratory was infested with ticks derived from dogs used in earlier investigations. It was of course possible that the dogs had already contracted infection from outside, but as they came from various sources and all sickened about the same time after admission this is not likely.

Infected dogs were first admitted to the laboratory about the beginning of October 1905, and shortly afterwards the walls were observed to be dotted over with gorged larvæ, nymphs, and adults. On the 22nd October 1905 newly hatched nymphs were seen and a little later adults.

Dog 5. Young pup—admitted 22nd October 1905.

26th October 1905. Many gorged nymphs evidently derived from the laboratory seen on dog.

27th October 1905. Dog very sick. Complete anorexia and prostration. Piroplasmata swarming.

28th October 1905. Dog died in the night with hæmoglobinuria.

Dog 6. Pup—admitted 22nd October 1905. No piroplasma.
28th October 1905. Scanty piroplasma.
29th October 1905. Many large round forms.
5th November 1905. Moderate infection with pear-shaped forms. Developed a mild infection.

Dog 7. Pup—admitted 23rd October 1905. Moderate if any ticks. In good condition. No piroplasma.
28th October 1905. Dog in good health.
30th October 1905. Quiet and off colour. A moderate number of large round forms seen.
31st October 1905. Many parasites.
1st November 1905. Large forms plentiful. Dog quiet but not very ill.

Dog 13. Pup—admitted 12th November 1905. Blood examination negative.
18th November 1905. Scanty piroplasma.

Dog 14. Pup in good condition and free from ticks. No piroplasma. Admitted 18th November 1905.
29th November 1905. Very ill; piroplasma abundant.

Dog 17. Pup in good condition. Admitted 21st November 1905. No piroplasma.
30th November 1905. Slightly indisposed. Piroplasma scanty.

Dog 18. Pup—admitted 19th November 1905. Extremely numerous nymphs and larvæ. No piroplasma.
26th November 1905. Abundant piroplasmata. Probably infected from nymphs picked up a few days before admission.

Dogs 24, 25, 26, 27 and others kept protected from ticks preparatory to feeding experiments did not develop infection, nor in later experiments did healthy young pups of this class develop infection when kept under protective conditions in the laboratory.

The general progress of the disease has been indicated; but the exact relationship of the parasite to the disease requires further discussion. Nocard and Motas note febrile periods characterised by the presence of numerous parasites which exhibit sign of amœboid activity and multiplication, and a subsequent period in which parasites are scanty and do not show amœboid movements.

They also speak of a hæmoglobinuric crisis. Other authors have paid little attention to periodicity in the disease, it being generally taken for granted that development of the parasite is irregular and devoid of periodicity.

During the examination of different cases it was often observed that immediately following an outburst of parasites there was a time during which parasites almost disappeared from the blood and that there followed, usually at an interval of about twenty-four hours, another outburst of parasites; and later observations have shown that there is in canine piroplasmosis a condition closely resembling

the periodicity of malaria in man. If examinations be made every few hours there will be found as a rule at the same time of the day, usually in the morning, a period during which parasites are very numerous, red cells infected with four, eight or sixteen forms and groups of free forms being conspicuous. Following such a period there occurs in the great majority of cases a time when parasites are few or even in some cases apparently absent from the peripheral circulation and when multiple forms are difficult to find. This gives place again to an increased number of parasites and the occurrence of another attack. As a rule the interval between two such attacks is, as previously stated, about 24 hours, but it is not infrequent to find what appears to be a double infection present, one attack taking place in the morning and another some time in the evening. In severe cases the periods of attack may run into one another giving rise to more or less continuous infection; but especially towards the beginning and end of the initial outbreak the cycles are short and separated by well-marked intervals during which parasites are scanty. In the chronic disease short attacks lasting a few hours occur, parasites in the intervals being extremely scanty.

It was thought at first that the periods represented a cyclical development of the parasite and that the formation of eight and sixteen forms was analogous to sporulation of the malarial parasite. But the result of counts of parasites already given showed that this view required considerable modification, especially when it was observed that at the critical period, when free forms became a conspicuous feature, these were derived not only from corpuscles containing large numbers of parasites, but also from corpuscles containing two and four forms.

The nature of the process seemed therefore to demand careful observation, and a number of cases were followed very closely throughout several days to determine what changes actually took place. That the number of parasites undergoes a great increase at the time of attack has already been noted, and the charts at the end of this memoir demonstrate its extent. It was also found that, though the relative frequency of the different forms remained in the main unaltered, there was at the time of crisis a distinct increase in the percentage of corpuscles containing four and more forms; this also will be apparent from the charts. In the case of the single and double forms distinct alterations in their proportions also took place, there being times when the single forms preponderated very greatly over the double forms, and other times when the latter equalled or even excelled the former in frequency. Unfortunately in the case of the single and double forms it has not been possible to demonstrate accurately the relation of the changes to the attack. On the whole a distinct impression was obtained that a large increase in the relative frequency of single forms together with an absolute increase of total parasites was usually the first sign of a coming attack,

and that as the attack progressed double forms became more frequent, often to yield, shortly after the crisis, to a relative increase in the single forms.

During the early periods of an attack free forms if present are rare, but at the crisis free forms lying in groups of two, four, eight or more are a striking feature.

At this time, therefore, one would expect that there would be a greater destruction of red cells than at other times, but my observations have as yet been too few to enable me to determine this.

With the course of infection changes in the blood both as regards the red and white corpuscles are considerable. The reduction in the red cells has already been referred to: some interest attaches, however, to the time at which destruction is greatest. This is undoubtedly during the first few days when attack after attack occurs and when for days together swarms of parasites occur in the blood. Very often the number of parasites is out of all proportion larger in one attack than in the others, and at such times parasites may number more than the red cells.

Changes in the leucocytes are also considerable. Nocard and Motas describe an increase in their number reaching in one dog to 54,000 per c. m. which they say is mainly due to an increase in the polymorphonuclear cells. J. A. Wright also notes that the number of leucocytes is usually greatly increased from the time parasites occur in the blood, the number in one of his dogs reaching 60,000. But in one case he notes a reduction.

An increase in the leucocytes has been observed by me in all the cases in which these cells have been counted; in addition there are changes in the leucocyte values. This consists of a marked increase in the large mononuclear elements and often in the transitional cells, extremely large cells being then present. The percentage of mononuclears is usually raised to 10-15 per cent. in place of the normal value of two per cent., but has reached in one count 26 per cent. The greatest increase is as a rule seen immediately before and at the crisis of free forms. At this period also phagocytosis is most intense and leucocytes are seen packed with more or less altered parasites.

To summarise; the course of a typical infection the result of subcutaneous inoculation of infective blood or the bite of pathogenic ticks is as follows:—

At a variable period after infection a few large forms are seen. A day later parasites are generally numerous, and about this time the temperature is raised; but the dog is not ill nor does it show any sign of anæmia. During the next two or three days parasites increase in numbers, being especially numerous during periods of variable duration characterised by an increased percentage of multiple-infected cells, the presence of numerous free forms, a mononuclear increase, and an intense phagocytosis. Young or very severely infected dogs usually die at about the third access, which is usually the most severe, very often

with hæmoglobinuria. Should the dog not die at this time or shortly afterwards, it either recovers or exhibits the so-called chronic type of the disease, which is characterised by a much reduced number of parasites, but in which, if the case be closely watched, parasites are seen at certain times to increase in numbers and after showing an outburst of multiple-infected cells and free forms to disappear quickly. In this form of infection the dog may die at almost any time, but as a rule eventually recovers, parasites being afterwards present for weeks.

EXAMPLES OF CASES.

Case 1, Dog No. 98.—Inoculated subcutaneously with infective blood on 22nd October 1906. Parasites were first seen on the morning of 26th October 1906 and were abundant on the morning of 27th October 1906 when the percentage of mononuclears had risen to 18. By the afternoon of 27th October 1906 parasites had fallen to less than one in ten fields of the microscope and the mononuclear percentage to 3. On the morning of 28th October 1906 only a few parasites were present but the mononuclears had risen to 5 per cent. The dog recovered after passing through an unusually mild attack. (*Vide* Chart I.)

Case 2, Dog No. 99.—Inoculated subcutaneously with infective blood on 22nd October 1906. Parasites were first seen on the morning of the 26th October 1906 and were numerous on the morning of 27th October 1906 when there was a mononuclear increase to 6·6 per cent. In the afternoon parasites numbered only an average of one in ten fields and the mononuclears had returned to the normal value of 2 per cent. On the morning of 28th October 1906, parasites were numerous and the mononuclear value was 16 per cent. (*Vide* Chart II.)

Case 3, Dog No. 136.—Infected by the bite of ticks on 10th December 1906. Parasites first seen on the morning of 18th December 1906 when they were very numerous, double and single forms greatly preponderating. Later in the day multiple-infected cells and free forms were abundant. Next morning parasites were reduced in numbers, but an outburst occurred on 21st December 1906. A high mononuclear value was present throughout the 18th and 19th. The dog died on 23rd December 1906 after an access, but at death parasites were only to be found with difficulty.

Case 4, Dog No. 139.—Infected by the bite of ticks on 10th December 1906. Parasites first seen on the morning of 18th December 1906 when they were very abundant; the mononuclears numbered 13·3 per cent. In the afternoon parasites were very scanty and the mononuclears formed only 3 per cent. of the total leucocytes. On the morning of 19th December 1906 parasites were abundant and the mononuclears had reached 26 per cent. In the afternoon of 19th December 1906 and morning of 20th December 1906 parasites were still more

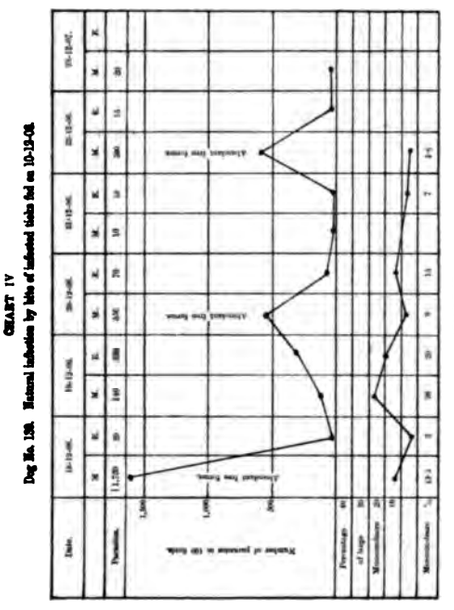


CHART IV

Dog No. 138. Natural infection by bite of infected ticks fed on 10-12-06.

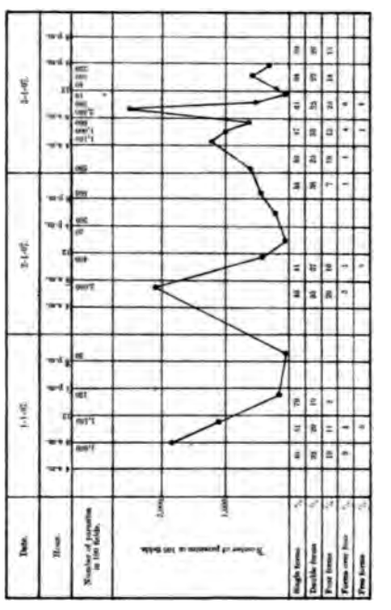


CHART V

Dog No. 146. *Subcutaneous inoculation on 28-12-06.*

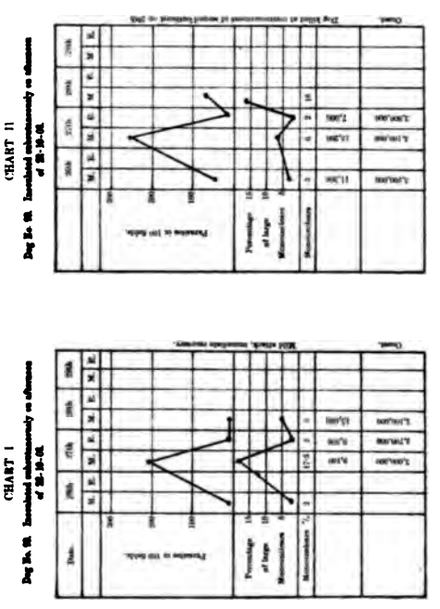


CHART I

Dog No. 68. *Inoculated subcutaneously on afternoon of 28-10-06.*

CHART II

Dog No. 69. *Inoculated subcutaneously on afternoon of 28-10-06.*

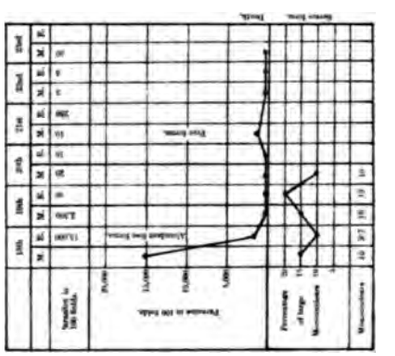


CHART III.

Dog No. 139. *Natural infection by bite of infected ticks fed on 10-12-06.*

CHART VI.

Dog No. 160. Inoculated subcutaneously on 10-1-07. Ten parasites counted in 100 fields on 15-1-07.

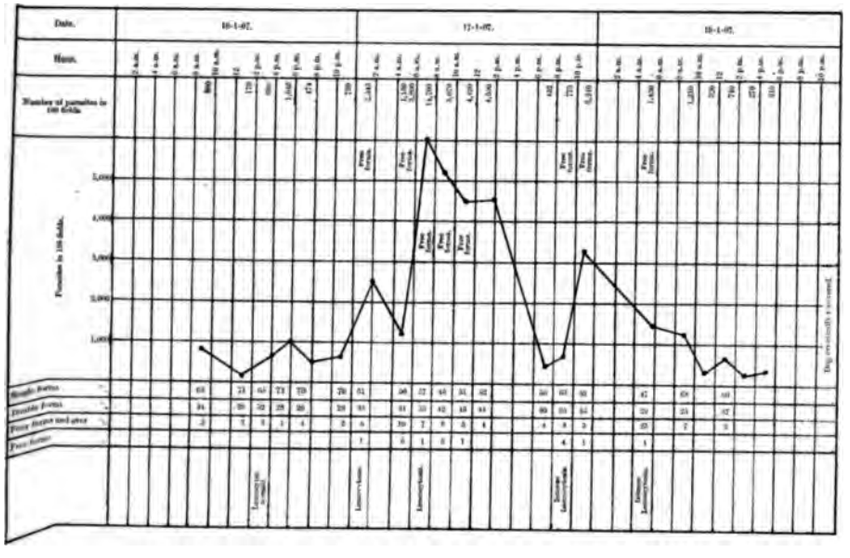
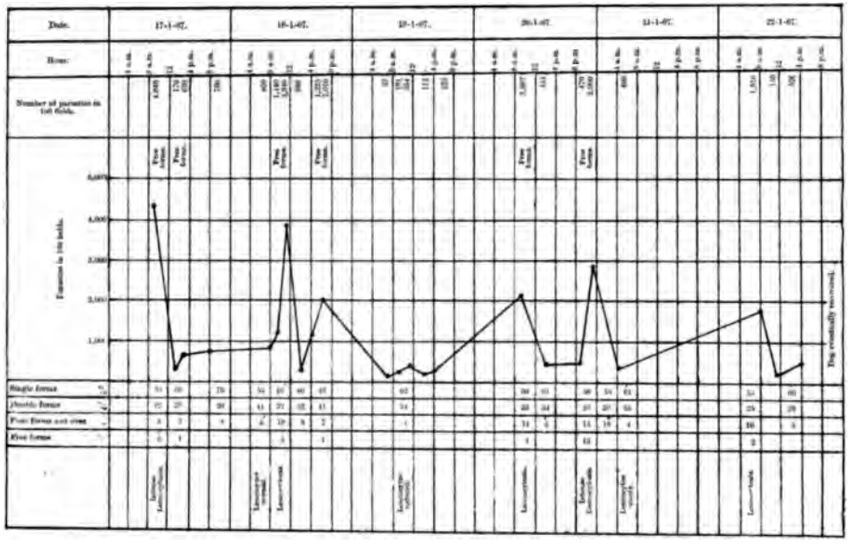


CHART VII.

Dog No. 162. Inoculated subcutaneously on 10-1-07.



PIROPLASMA CANIS AND ITS LIFE CYCLE IN THE TICK.

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Helio., S. I. O., Calcutta.

numerous. On the afternoon of 20th December 1906 their number was greatly diminished and remained low until the morning of 22nd December 1906, when another access occurred, ending in an extremely sudden reduction in the number of parasites.

In this and other examples given examinations were made only two or three times a day and it is possible that in some cases accessions of parasites and periods of disappearance passed unnoticed. In the next three cases given in Charts V, VI and VII, examinations were made every two or three hours and in several instances throughout the night. In dog 146 a quotidian cycle is present. In dog 150, in which the disease was of a very severe type, the periods of access are extended over long periods and there appeared to be two accesses in the twenty-four hours. In dog 152 a quotidian access is apparent each morning, but a second less pronounced access was present in the afternoon especially noticeable on 18th December 1906. As the charts give the observations made it is not necessary to refer to them in detail. The number of parasites is the number present in one hundred fields counted as far as possible in corresponding proportions of films of similar character. Whether free forms or multiple-infected cells are seen depends very much upon the exact stage at which the film happens to be taken, an hour or even half an hour sufficing to give changed conditions in their relative proportion. (*Vide* Charts I to VII.)

III.

THE TRANSMISSION OF PIROPLASMA CANIS BY TICKS.

Dog Ticks.

The evidence in favour of transmission of the piroplasmata by ticks has already been briefly summarised. In the case of *Piroplasma canis* the evidence is particularly convincing, though there is still much to be learned of the details connected with its spread in different parts of the world.

Fig. 2.
Probable geographical distribution of *Rhipicephalus sanguineus* (Latreille).

On account of the analogy with Texas fever and because they found dogs infested with *Ixodes reduvius*, Piana and Galli Valerio thought that this species might be the transmitter. Similarly Almy, and Nocard and Motas believed that *Dermacentor reticulatus* can transmit the disease; but actual experimental demonstration of transmission of the disease in Europe is still wanting, due very much, it would seem, to a want of knowledge by the earlier observers of the life history of the ticks with which they were dealing.

The first to infect dogs experimentally by means of the bite of ticks was Lounsbury, who in a series of experiments lasting over three years proved beyond all doubt that *Piroplasma canis* in South Africa is transmitted by the common dog tick of that country, *Hæmaphysalis leachi*.

But *Hæmaphysalis leachi* is unknown in Europe and has not been found in

Madras. It is therefore certain that other species of tick are also concerned, and the geographical distribution of ticks found commonly enough on the dog to be called dog ticks is important. Our knowledge on such lines is unfortunately meagre, but recorded * observations would indicate that those species of ticks found with any frequency on the dog are the following:—

Hæmaphysalis leachi (Audouin).—This is an African species reported from Egypt, East Africa, Algiers, Sierra Leone, French Congo, Transvaal, Cape, and Mozambique. Neumann, however, mentions two specimens taken from a tiger in Sumatra. Careful search for this tick in India has been without result though a species somewhat resembling it has been found on the mongoose. It certainly does not occur as a dog tick in Southern India.

From Lounsbury's work it is evident that this is the dog tick of South Africa. Other ticks are, however, found on dogs at the Cape, notably *Ixodes pilosus* and *R. capensis*. At Nelspruit, in the Transvaal, dogs were found to carry more *R. simus* than the ordinary species and specimens of *R. appendiculatus*, *A. hebræum*, and *Hyalomma ægyptium* were also found on them. Experimentally also Lounsbury has reared *R. decoloratus* on dogs and the early stages of *A. hebræum* and *R. evertsi* may feed to repletion. So far nothing is known of the pathogenicity of these species.

D. reticulatus (Fabr.).—Has a wide distribution, being recorded in Europe from France, Portugal, Roumania, and England; in Asia from Caucasus, Japan, Turkestan, Siberia, and Persia; and in America from California, Texas, and New Mexico.

Ixodes reduvius (Linné).—A species common on dogs. Occurs over the whole of Europe, the nymph and larvæ being found on lizards, birds, and squirrels, etc. Also in Algeria, Caucasus, and in the United States, being recorded from Baltimore, Carolina, Florida, California, Kansas, and Texas.

Ixodes hexagonus (Leach).—Common on dogs in Europe; also upon various animals, the dog not being noted as a host, in Texas, Kansas, Maine, New York, Iowa, and Colorado. Nymphs and larvæ occur on small animals and birds.

R. sanguineus (Latreille).—Has been taken from the dog less often than from other animals. Neumann mentions it as pre-eminently the dog tick, and in India with one exception (in which dog ticks sent to me were unfed *H. ægyptium*) they have always proved to be *R. sanguineus*. The distribution of this tick is extremely wide; in Europe it is recorded from France, Italy and Sicily, Corsica and Roumania; in Africa from Egypt, Abyssinia, Zanzibar, Senegal, Algeria, and Tunis; in Asia from Persia, China, Malay, and India; it is also reported from America, the Philippines, Oceania, and Australia.

* *Vide* chiefly Neumann, 215—217.

As this species carries *Piroplasma canis* in India it would appear in itself sufficient to account for infection over a great part of the globe.

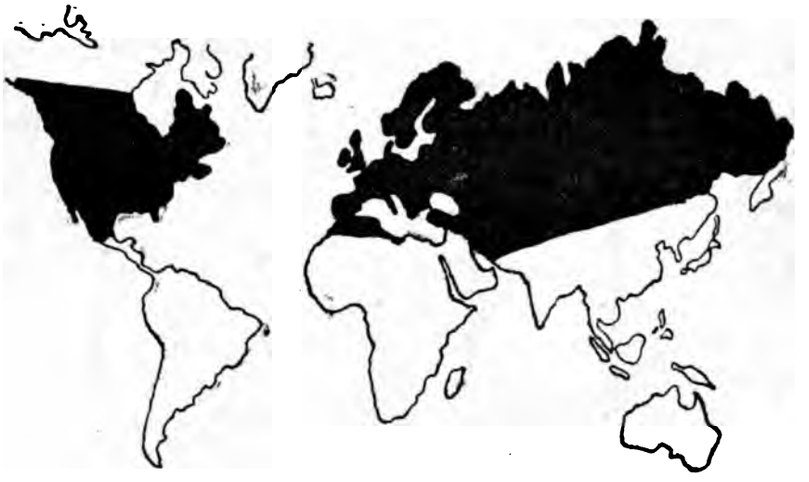

FIG. 3.

Probable geographical distribution of *Ixodes reduvius* and *Dermacentor reticularis*.

R. bursa (Canestrini and Fanzago).—Also frequently found on the dog, but much more frequently on other domestic animals. In Madras it is much less adapted for life upon the dog than *R. sanguineus* and is seen chiefly as a parasite of the goat. It is recorded in Africa from Algiers, Senegambia, East Africa, West Africa, Kilimanjaro, Daresalam, the Congo, the Cape, and Abyssinia. Experiments with this species in Madras failed.

Dermacentor Americanus (Linné).—Frequent on the dog. Recorded from Maryland, Texas, California, Nantucket, Labrador, Minnesota, Alabama, Colorado, Florida, Kansas, Alaska, and Mexico.

Amblyomma cajennense (Koch).—Frequent on the dog. Recorded from Brazil, Cayenne, Mexico, Central America, Cuba, and Vera Cruz.

A. striatum (Koch).—Found on the dog. Brazil, Batua, Marago.

A. calcaratum (Neumann).—Found on the dog. Brazil.

Ixodes ovatus (Neumann).—In Japan and Amoor, on the horse, hare, and dog.

Ixodes holocyclus (Neumann).—Australia and India, on the dog and sheep.

Other species are recorded from the dog, but do not appear to frequent this host as a rule, such as *H. ægyptium*, a species most frequent on the ox, dromedary, and camel, and in Algeria on the horse, mule, and ass, occasionally also on the sheep, stag, dog, cat, and man. It is improbable that these are to any extent concerned in the spread of canine piroplasmosis, although it is possible some of them might transmit the disease experimentally.

Of those specified *R. sanguineus* is probably the species having the widest

distribution and would in itself serve as a transmitter in Southern Europe, North Africa, and much of the East. For South Africa there is a special dog tick, *H. leachi*. In Europe and more temperate regions the various species of *Ixodes* seem to act as carriers. In Japan the only dog tick noted is an *Ixodes* and an *Ixodes* is found on dogs in Australia. In Central America several species of *Amblyomma* seem to be associated with the dog.

Ticks of Madras.

In Madras the common ticks are *Hæmaphysalis flava* (Neumann), *H. spinigera* (Neumann), *Rhipicephalus sanguineus* (Latreille), *R. bursa* (Can. et Fanz.), *R. simus* (Koch), *R. annulatus* (Say), *Aponomma gervaisi* (Lucas), and other species on reptiles, various species of amblyomma on pigs, cattle, etc., and *Hyalomma ægyptium* (Linné).

R. simus I have found only in the hill districts (Nilgiris) on cattle, especially the buffalo. *H. spinigera* occurs on the tiger and panther on the lower slopes of the hills. The others are all more common on the plains. Cattle rarely shew other species than *R. annulatus* and *H. ægyptium*. The commonest horse tick is *H. ægyptium*, which is also found on goats and sheep. In Northern India this tick infests the camel, and I have received specimens sent as dog ticks. *H. flava* is the common tick of the goat, sheep, and buffalo. It is found on dogs when these are used for herding goats and sheep, and the same or a closely related tick is found on the cat. *R. bursa* is also most common on the goat and sheep, and though it will go through all its metamorphoses on the dog it is not often seen naturally in this situation. *R. sanguineus* is, in contradistinction to all these, the dog tick; it is found in considerable numbers on almost every dog, but is very rarely found on other hosts.

The different species of tick mentioned differ very markedly in their appearance, their habits, and even in the general aspect of the immature stages. *R. bursa* is a larger tick than *R. sanguineus* and becomes so completely distended as to resemble an oval bladder, whilst *R. sanguineus* after it has dropped from its host after engorgement is somewhat flattened and the dorso-ventral muscles cause deeper indentations and puckerings than in *R. bursa*. *H. flava*, which in the unfed condition is a much smaller tick than either of the above, becomes when distended nearly equal in size to *R. sanguineus*, the cuticle of the belly becoming so stretched that the dark colour of the blood shews through, giving the fed tick the appearance of a black currant.

R. sanguineus and to a less degree *R. bursa* when gorged climb upwards, but *H. flava* drops to the ground. The result is that the adult females of *H. flava* are apt where dogs are kept in cages to be removed in the tray with the urine and fæces, or to be swept away in the course of cleaning up. It is possibly

partly on this account that, although large numbers of *H. flava* have from time to time been imported into the laboratory, the progeny have scarcely ever been seen; but even when the adults are placed in cages from which they cannot escape the result is identical. *H. flava* is not a dog tick, nor is it adapted for living upon dogs; and the same remark applies in a lesser degree to *R. bursa*, which also for some reason tends to disappear when introduced among dogs. Experiments in Madras have therefore been conducted mainly with *R. sanguineus*, which has been found to transmit the disease.

General Life History of Ticks.

Before approaching the question of the part taken by *R. sanguineus* in the transmission of *Piroplasma canis* a brief summary of the life history of ticks is necessary. All members of the scutum bearing ticks undergo after hatching from the egg two metamorphoses, namely, that from the larva to the nymph and that from the nymph to the adult. The larvæ are hexapod with a shield covering the anterior portion of the dorsum and have the skin of the posterior portion thrown into innumerable fine folds to allow of the great expansion which takes place during engorgement.* The nymphs are octapod and resemble in general appearance minute adult females. They also have a scutum anteriorly and a plicated integument over the posterior portion.* At each stage the tick gorges itself with blood, and the increase they undergo in size at this time brings them approximately to the size of the next stage.

Each immature tick becomes motionless after feeding and remains for a considerable period as incapable of movement as the pupæ of insects.

The final metamorphosis results in the formation of sexually differentiated or adult ticks. The dorsum of the female like that of the larva and nymph is covered only in its anterior portion by a scutum and the integument over the posterior portion is plicated like that of the larva and nymph to allow of the extreme distension which takes place at engorgement. The male, which does not increase in size after feeding, possesses a scutum covering the whole dorsum.

Unfed adult ticks measure about 2 to 3 mm. in length, but small species, especially the males, may measure considerably less, and large species such as *A. hebræum* may measure 5 mm. or more. When fully gorged, the female tick reaches a length of from 6 to 10 mm. or more. The males are generally found associated with a female, and Lounsbury notes that in *H. leachi* the males

* Allen [190] in his recent work on the anatomy of the tick describes this condition minutely and notes that the outer portion only of the cuticle is plicated.

move their position to find mates. On the other hand in *A. hebræum* the female is attracted by the male and will not attach herself except in his immediate neighbourhood. The males in most species remain on the host after the gorged females have dropped off.

The different species often shew a marked predilection for certain sites of attachment. Thus Lounsbury notes that the larvæ of *R. evertsi* are found always deep within the ear, whilst the adults choose the regions about the anus, and that *H. ægyptium* fixes itself upon the scrotum and groin. In India *R. annulatus* attaches itself to cattle almost anywhere on the belly, whilst *H. ægyptium* as in South Africa selects the groin. *H. flava* when upon goats and sheep is almost always upon the ears.

The behaviour of the gorged female prior to laying her eggs, and the position in which these are laid, differ in different species. Most of the cattle ticks when gorged crawl to the nearest shelter or bury themselves in the ground. *H. leachi* is described by Lounsbury as hiding itself among rubbish. The female *R. sanguineus* climbs upwards and lays her eggs by preference in cracks, squeezing herself into these so tightly that it is often impossible to remove her intact.

The eggs of the *Ixodidæ* are always very numerous. As a rule each female lays about 4,000 eggs, but in the case of large ticks the number is greater, reaching in *A. hebræum* 10,000 to 20,000. (Lounsbury.)

The time taken in hatching and the duration of the different stages vary greatly in different species and at different temperatures. According to Smith and Kilborne the eggs of *R. annulatus* in the States take from 15 to 18 days to hatch in very hot weather and 40 days in colder seasons. In the case of *H. leachi* Lounsbury notes the time of hatching at the Cape to be from 5 to 7 weeks in summer and 12 to 16 weeks in winter. Lounsbury notes the time of hatching of the eggs of the Bont tick to be eleven weeks in summer and many months in winter. The changes from larvæ to nymph are more rapid. In *H. leachi* the larva and nymph feed and drop from the host in from 48 to 75 hours. In summer the gorged nymphs hatch into adults in 18 to 20 days. It is interesting to compare these figures with those given later for *R. sanguineus* in Madras, where except during one or two months the climate is tropical.

Most important variations from the point of view of disease transmission occur in the relation of ticks in their various stages to different hosts.

The only group known to pass all the stages on the same host are the *Boophilus* group of the *Rhipicephalidæ*. In these the time for the changes are very short, being given by Smith and Kilborne as about a week in each case.

Many ticks drop from their host at each stage to undergo their metamorphosis. This habit was first described by Lounsbury in the case of *H. leachi*, but it has since been described by him in the case of *R. appendiculatus*,

R. capensis, *R. simus*, *A. hebræum*, *A. variegatum*, and *Ixodes pilosus*, and it holds good in *R. sanguineus*, *R. bursa*, *H. flava*, and two species of *Aponomma* common on reptiles in Madras.

Two species, *R. evertsi* and *H. ægyptium*, are described by Lounsbury and Theiler as remaining throughout the first two stages on the same host.

It appears that it is the usual habit of ticks to leave their host at each stage and that the passing of one or more metamorphoses on the same host is a special adaptation.

In the different stages a host of the same or a different species may be selected. *H. leachi* and the Indian dog tick attach themselves in all their stages to the same host, which may often be the same dog, and *R. appendiculatus* behaves similarly upon cattle. Other species feed as larvæ or nymphs on different hosts to those selected by them as adults, and in *I. hexagonus* and *I. reduvius* in Europe the nymphs and larvæ are found on lizards, birds, and upon small mammals such as squirrels, moles, etc. As a rule the immature ticks are less particular in their choice of hosts than the adult, but in certain South African species Lounsbury has found the larvæ very difficult to satisfy in this respect and he suggests that they normally choose some particular host at this stage.

As the genus *Rhipicephalus* is that to which the Indian dog tick belongs it will require some mention. Many of the species of this group have so close a general resemblance that their differentiation requires great care and the question of two or more species being present on dogs in Madras has been considered. The genus *Boophilus* created by Curtice for *R. annulatus* though not considered by Neumann in his memoirs as sufficiently distinct to be maintained as a separate genus undoubtedly contains a group of the *Rhipicephali* very distinct from those ticks of which *R. sanguineus* may be taken as the type. Mr. Lounsbury makes the remark that Neumann has since considered the separation of this latter group as the *Eurhipicephali*, a distinction which has much to recommend it.

Variation in size is considerable, but this is frequent in ticks and may depend upon the food supply in the larval or nymphal stage. It is also dependent upon the state of the tick, for a male whose vasa differentia are swollen with spermatozoa produces the impression of being a much larger tick than one in which these structures are empty. The apparent size of an unfed female also varies according to the period of fasting.

In *R. appendiculatus* and some other ticks a tail appears after the tick has fed for a certain time. A slight prominence may sometimes be seen in males of the Madras dog tick, but it rarely is sufficient to deserve the name of a tail nor does it approach in character that figured by Lounsbury in *R. appendiculatus*.

In spite of certain slight variations among my specimens I have been unable to satisfy myself that there is more than a single species which corresponds with Neumann's description of *R. sanguineus* (Latreille).

R. SANGUINEUS (LATREILLE).

R. sanguineus drops from its host after engorgement in each of its three stages, and whether in these it attacks the same or different dogs is determined by chance.

The fully replete female after dropping from the dog at once proceeds to crawl away, climbing, if it can, upwards sometimes to a height of over fifteen feet from the ground. Sooner or later it inserts the anterior portion or the whole of its body into some crack. Very often the crack is an extremely narrow one, much narrower than one would suppose it possible for the tick to enter; yet the ticks will be found firmly wedged within it having become very flattened in the process. The ova are laid by preference in some such situation, and where ticks are kept in captivity in tubes with crumpled paper they always choose a place to deposit their eggs where the paper lies against the glass or against another piece of paper, the eggs being deposited under these circumstances in a more or less flat sheet. The period of oviposition commences a few days after the female has dropped from the host, and the eggs are passed out continuously until the tick (shrivelled to one-third or one-quarter of its original size) eventually dies. Oviposition takes as a rule about four to seven days. The larvæ hatch in three or four weeks and crawl away, leaving the mass of empty egg-shells behind. By degrees the larvæ collect into swarms, which at first sight resemble masses of ova. These swarms are often of very great size and must be composed of many thousands of individuals. In nature the larvæ collect near the bottom of walls and when a dog brushes against this spot the larvæ transfer themselves *en bloc* to the dog. The larvæ will not feed immediately they are hatched, but require a period of some days' rest; hungry larvæ placed on a dog become gorged and drop off in from three to four days, but some drop even earlier. The larvæ care very little where they attach themselves, but usually do so on the hairy parts of the body where they are not easily detected. The gorged larvæ, which are little black seed-like bodies measuring about $\frac{3}{4}$ millimetre, crawl away and secrete themselves in cracks. For a day or two if the gorged larvæ are disturbed they crawl about, but eventually become quite inert and apparently dead. After about 9 or 10 days the skin breaks posteriorly and the nymph emerges.

The nymphs are light brown active creatures about the same size as the gorged larvæ and except that they are only about one-fifth the size there is a distinct general resemblance, even in the details of the rostrum, to the adult

tick. The nymphs crawl about and eventually find a host; they often are seen in little clusters, but never in such number as are the larvæ. They attach themselves chiefly to the skin of the body and may be seen and studied on the bare skin of the belly. When fully gorged they are a repetition on a larger scale of the gorged larva, and a forecast on a smaller scale of the gorged female adult. They fall off and enter cracks, and like the larvæ become apparently lifeless.

The nymphs in this state can be collected and handled like seeds, to which they have a strong resemblance. As they reach the time of eclysis the front portion becomes of a dead white colour. A little later, about 15 days after they have left their host, the nymphal skin cracks posteriorly and the adult hatches out.

Both males and females hatch out about the same time. They are at first quite soft and do not attach themselves for some days; then they get on portions of straw, etc., and wait for their host with the front legs extended in the air. As a rule the adult attaches itself to the ear and even quite deep in the meatus. It is especially fond of a little pocket on the posterior border of the ear. They also select the skin between the paws or that at the back of the neck. They may, however, attach themselves to almost any part of the body.

Adult females take from a few days to a week or more to become fully gorged and to drop off. The sexes attach themselves close together, the male lying beneath the female and copulation no doubt takes place when so situated. For the first 24 to 48 hours after attachment but little increase in size of the female takes place; usually, however, by the end of the second or third day a distinct increase in size is noticeable, though the general colour of the tick is still a dark brown. As the posterior portion of the female still further enlarges it becomes a buff colour and eventually a slaty gray. When fully replete the female measures 6 to 8 mm. in length.

The body of the tick is at first more or less uniformly distended, but after some days when the eggs have accumulated in the extremely long oviducts the anterior portion of the body is much swollen whilst the posterior parts are flattened. During the first days after leaving the host the female is restless and wanders about passing considerable quantities of the white malpighian secretion. Once having settled down to oviposition she rarely again moves, or if disturbed does not travel far.

The ova are lighter in colour than those of *H. flava* and more numerous, but they are difficult to detect from those of many species of ticks. They number some thousands and adhere loosely together on account of an oily covering which they receive from the " cephalic gland " described by me in a previous publication—a structure whose function I find had been already ascertained by Lounsbury.

The changes undergone at the metamorphoses are all important with reference to the development of the parasite and must be briefly referred to in order to make intelligible the passage of piroplasma through the tick.

For the first twenty-four hours or somewhat longer after leaving the host both larval and nymphal stages are active. At this time the organs are practically those of the unfed condition with the exception that the diverticula are enormously distended with blood. After several days have passed the cuticle becomes thicker and denser and the ticks exhibit no sign of life ; but changes in the tissues consisting in the formation of masses of undifferentiated embryonic tissue are proceeding rapidly. After a certain time this embryonic tissue forms a rough model of the future nymph or adult as the case may be, and this, lying as it does loosely within the hard and rigid cuticle of the previous stage, can with suitable manipulation be turned out upon the slide. In it can be made out indications of the limbs, rostrum and other parts, all modelled in undifferentiated tissue consisting of polygonal cells of about the same size and appearance. Little by little the model becomes more exact and the cells differentiate to form the cuticle, muscles, and organs of the future stage. It is necessary only to follow in detail the formation of the salivary glands of the adult, since the changes undergone in the larva are similar, but on account of the difficulty of manipulation have not been followed so closely. In the embryonic adult each of the future limbs is seen as a bud-like outgrowth merging at the base into a dense mass of embryonic tissue. Quite early intercellular streaks which stain a reddish colour with Romanowski stain appear. These are the rudiments of the future intraglandular salivary ducts and they spread further and further among the tissue cells as development proceeds. Closely applied to them are seen cells which have smaller and darker staining nuclei than the rest of the tissue (Pl. III, fig. 7, lower nucleus). These eventually form the ducts of the gland. The acini are formed from cells lying at the side of the embryonic ducts (Pl. III, fig. 7), each acinus originating from a cluster of cells which arrange themselves regularly to form an embryonic acinus (Pl. III, figs. 2 and 3). In most of the acini all these cells develop equally forming a multicellular acinus (fig 2), but in the case of certain acini lying along the main ducts one cell takes on growth out of all proportion to that of the rest resulting in the single celled " poison " acini seen in the adult gland (figs. 3 and 9).

Of the apparently similar cells forming the mass of embryonic tissue at the bases of the embryonic limbs some may be progenitors of cells of the salivary acini whilst others eventually become muscular, fatty or tracheal tissue.

In the larva the salivary gland consists of only a few acini, some being of the ordinary type and others of the poison type. In the nymph many more acini exist, and in the final change a great portion of the embryonic tissue at the base

of the legs becomes converted into salivary tissue, the salivary glands in the adult occupying a comparatively enormous area in the body of the undistended tick. The chance that any cell of the original embryonic tissue may in whole or in part end by becoming salivary tissue is therefore considerable, and any parasite embedding itself in one of these cells stands a very fair chance of eventually finding itself in a salivary acinus. As will be seen later piroplasma makes still further provision to this end.

IV.

EXPERIMENTAL INFECTION.

The first successful investigations regarding the transmission of *Piroplasma canis* by ticks were those of Lounsbury, who found that, though canine piroplasmosis, like Texas fever, is transmitted through the ovum, it is not induced by the bite of larvæ hatched from ova of infected mothers, but by these only after they had gone through two further metamorphoses and had reached the adult state. These experiments of Lounsbury are so conclusive that there is little necessity to consider them in detail. Experiments were also undertaken by Lounsbury to see whether infection taken in at one stage of metamorphosis could be transmitted at a later stage, as happens in the case of East Coast fever when transmitted by *R. appendiculatus*. These experiments led Lounsbury to conclude that such stage to stage transmission does not take place ; but his experiments in this respect are not so numerous or so conclusive as those connected with hereditary transmission. Infection was later experimentally induced by Nuttall in England by means of infected ticks (*H. leachi*) sent to him by Lounsbury from South Africa, a demonstration of a striking kind that canine piroplasmosis is a tick-borne disease.

My own experiments have shewn that the tick *R. sanguineus* (Latreille) is also capable of transmitting canine piroplasmosis and a demonstration similar to that which has been given in the case of *H. leachi* also exists, since ticks (*R. sanguineus*) taken home by Captain James, I.M.S., gave rise to a severe infection in a dog at the Lister Institute.

As in Lounsbury's experiments the hereditary method of transmission is placed beyond doubt. Unfortunately conclusive experiments with regard to stage to stage infection have not yet been carried out and the evidence for this method of infection is at present based only on the microscopical observations given in the next section.

My earlier experiments shewed that infection resulted from the bite of adults, but they were not sufficiently closely watched to enable accurate deductions to be drawn from them. This was rectified in later experiments given here where every possible precaution was taken to exclude error and to follow the details of the process.

The cages used were of iron and immediately before use they were plunged into a vat of boiling water. The tables used were flooded with scalding water

and every crack thoroughly treated. In the laboratory the table legs were placed in shallow trays filled with kerosene oil and separate tables were used for every experiment.

The dogs used were of the best breed obtainable. Very young dogs though advantageous in some respects were apt to die from other causes than piroplasma and pups of medium size were therefore for the most part utilised. Before being used in the experiments the dogs were quarantined in newly dipped cages for a period of five days in order that any larvæ or nymphs they might have upon them should drop off. The dogs were then removed to a fresh cage until required. Control dogs from the litters used in the experiment were in a proportion of cases kept under observation.

During the experiments the cages were kept surrounded with a muslin sheet in each case fresh from the wash to enable fed ticks to be observed and collected.

In some of the experiments the conditions were arranged to resemble what takes place in nature, the sequence of the metamorphoses not being interfered with. Under these circumstances cages became infective, every susceptible dog placed in such cages contracting the disease. In others isolated feeding experiments were made.

Series A.

Experiment 1.—A batch of seven adults were collected from dog No. 97 in the early stages of an attack of piroplasmosis. Of these five were dissected, four shewing club-shaped bodies in the ovaries. The larvæ from ova laid by some of these and by the two remaining adults were on 25th October 1906 liberated in cage 5 containing dogs Nos. 100, 101, 102, 103 and 104, and to these dogs the larvæ quickly attached themselves, fed larvæ being first seen on 27th October 1906. On 27th October 1906 dogs Nos. 101, 102 and 104 were removed to a fresh cage and kept under observation. They all died from malnutrition on the dates noted in the table, but lived long enough to shew that the bite of the larvæ had not caused infection up to the 16th day. Dogs 100 and 103 died about the same time, no piroplasmata being found or any sign of infection.

On 27th October 1906, dogs 111 and 112, both from the same litter, were placed in the cage and were bitten by some of the larvæ which had not yet fed. On the 4th November 1906 fully hatched nymphs derived from the original larvæ were first seen and gorged specimens were abundant on 7th November 1906. So that nymphs almost certainly attached themselves to both dogs on the 4th or 5th November 1906. Dog 111 died on 10th November 1906 and dog 112 on 11th November 1906 with abundant piroplasma. These being

young dogs probably died 24 to 48 hours after the first appearance of parasites, making the incubation period, supposing they were infected by the larvæ, about 13 days, or, as appeared to be the case if infected by the nymphs, 5 days. In spite of the shortness of the latter mentioned time and the difference between the time of incubation and that given by other observers it is probable, as will be seen from later experiments, that it was the nymphs that gave rise to the infection with a short incubation period. In the salivary glands of rather a small proportion of nymphs from this cage the bodies shewn in Pl. III, fig. 6, and Pl. II, fig. 30, and described later were at this time seen.

On 13th November 1906 dogs 121 and 122 were added to the cage. They were at once attacked by nymphs which had not yet fed. On 16th November 1906 the blood was negative. On 17th November 1906 dog No. 121 had scanty piroplasma and, 122 was negative. On 20th November 1906 dog 121 died of the typical acute disease with hæmoglobinuria. Dog 122 on this date was still free from piroplasma. On the 22nd November 1906 numerous adults which had hatched from the nymphs were observed and some had attached themselves to dog 122. On 28th November 1906 dog 122 shewed a scanty infection afterwards developing a very severe form of the disease with paralysis of the hind legs and hæmoglobinuria and dying on 30th November 1906. In this case the number of nymphs available to feed on Nos. 121 and 122 were not very many, and No. 122 appears to have escaped infection from this source, but to have succumbed to infection derived from the numerous adults in whose salivary glands parasites were detected by microscopic examination.

Control dog 113 of the same litter as 111 and 112 but not used for experiment remained free from piroplasma.

Experiment 2.—Two dogs, Nos. 127 and 129, from a litter of four, the other two dogs, 128 and 130, being kept as controls, were placed in a cage on 29th November 1906 with adults removed from cage 5. On 4th December 1906 both shewed abundant piroplasma, but eventually recovered after a mild attack. Controls 128 and 130 up to and on 27th December 1906, on which date they were destroyed, shewed no piroplasma.

Experiment 3.—Two dogs, 135 and 136, were placed in a cage on 10th December 1906 along with adults collected from cage 5 kept up to this time in test tubes in the laboratory. On 18th December 1906 both dogs were observed to be ill and extremely numerous parasites were found in the blood of both. Dog 135 died on the same day and 136 some days later.

In the series of experiments both nymphs and adults undoubtedly gave rise to infection. The experiments as regards the non-infectivity of the larvæ were not, however, so conclusive as could be wished owing to the dogs used at the beginning being too young.

SERIES B.

Experiment 4.—A number of adults were collected on 18th September 1906; from dog 90 four days after large numbers of parasites had been seen in the blood. The larvæ hatched out from ova laid by these were placed on dogs 109 and 110 in cage No. 3. On 4th November 1906 newly hatched nymphs were observed and gorged nymphs were seen on 7th November 1906. On 12th November 1906 both dogs shewed swarming piroplasma, dog 109 dying on the 15th November 1906 and dog 110 being killed the same day. The same remarks apply to these dogs as to 111 and 112 in experiment No. 1.

On 13th November 1906 dogs 105 and 107 from a litter of which the other two dogs, 106 and 108, were used in experiment No. 6 were added to the cage. Both dogs, but especially dog 107, were attacked at once by nymphs, in the salivary glands of about half of which parasites were present. On 16th November 1906 dog 107 had scanty piroplasma and on 17th November 1906 both were infected. Dog 107 died on 19th November 1906 with paralysis of the hind legs and hæmoglobinuria and dog 105 recovered. A few adults were first seen in the cage on 14th November 1906. The other dogs of the litter, 106 and 108, used in the experiments with *R. bursa* remained free from infection. The incubation period following the bite of the nymph in this case was four days.

On 11th December 1906 dogs 131 and 132 were placed in the cage. On 21st December 1906 both dogs were observed to have pallid tongues and piroplasmata were found in their blood.

Experiment 5.—On 10th December 1906 two dogs, 139 and 140, from a litter of five healthy dogs of large English breed, of which 137 and 138 were kept as controls, were placed in a cage with adults hatched from gorged nymphs collected from cage 3. On 18th December 1906 both shewed a rise of temperature and numerous piroplasmata were present in the blood. The control dogs 137 and 138 remained free from infection for many weeks.

Experiment 6.—An experiment on similar lines to Nos. 1 and 4 was carried out with larvæ from adult *R. bursa* which had been fed on dog No. 86 when parasites abounded in the blood. On 25th October 1906 dogs 106 and 108 were placed in cage 6 with very numerous larvæ. On 28th October 1906 two other dogs, 118 and 119, were added to the cage. All the dogs were bitten by nymphs of *R. bursa*, fed nymphs being seen in immense numbers. On 20th November 1906 many adult *R. bursa* had attached themselves. None of the dogs became infected throughout the whole course of the experiment.

These experiments shew conclusively that infection is passed on by the mother tick to her progeny and that this infection is transmissible by the nymphs.

As regards the continuance of the power of transmission to the adult one cannot be so dogmatic for the following reasons. In *R. sanguineus* it is almost certain from evidence given later that stage to stage transmission as well as hereditarily transmitted infection takes place. Dealing then with so short an incubation period the possibility of reinfection of at least some of the nymphs and the passage of infection by this means to the adult cannot without special precautions, not taken in these experiments, be rigidly excluded. In the experiments of series B such a reinfection almost certainly took place and it cannot be excluded in others. It is, however, from the nature of the development of the parasite quite reasonable to suppose that a continuance of infectivity from the nymph to the adult may take place.

Again as regards the larva the experiments are too few to determine with certainty that the larva never transmits. That it did not do so in the heavily infected ticks used in the present experiments is, however, suggestive that it is, as in *H. leachi*, non-infective.

Further feeding experiments to determine a number of details are necessary, but since the main facts for the demonstration of which they were designed are quite clear and since they were undertaken mainly to enable observations to be made upon the parasite they will for the present suffice.

TABLE III.—*Shewing in tabular form the results of feeding experiments.*

Litter.	Number of Dog.	Species of tick.	Stage of tick.	Date on which ticks were added to cage.	Date on which infection was first noticed.	Incubation period in days.	REMARKS.
No. 1	100	R. sanguineus	Larvæ	25th Oct. '06	.....	...	No infection on 7th November 1906 or 13 days after being bitten.
No. 1	101	Do.	Do.	25th Oct. '06	.....	...	No infection on 8th November 1906 or 14 days after being bitten.
No. 1	102	Do.	Do.	25th Oct. '06	.....	...	No infection on 10th November 1906 or 16 days after being bitten.
No. 1	103	Do.	Do.	25th Oct. '06	.....	...	No infection on 8th November 1906 or 12 days after being bitten.
No. 1	104	Do.	Do.	25th Oct. '06	.....	...	No infection on 9th November 1906 or 15 days after being bitten.
No. 2	105	Do.	Nymphs	13th Nov. '06	17th Nov. '06	4	Mild attack. Piroplasma in blood.
No. 2	106	R. bursa	Larvæ, nymphs and adults.	25th Oct. '06	.....	...	No infection resulted. Destroyed after some weeks.
No. 2	107	R. sanguineus	Nymphs	13th Nov. '06	16th Nov. '06	3	Severe attack. Piroplasma in blood.
No. 2	108	R. bursa	Larvæ, nymphs and adults.	25th Oct. '06	.....	...	No infection resulted. Destroyed after some weeks.
No. 3	109	R. sanguineus	Larvæ	25th Oct. '06	.....	...	
		Do.	Nymphs	4th Nov. '06	12th Nov. '05	8 ?	Acute attack. Piroplasma in blood.
No. 3	110	Do.	Larvæ	25th Oct. '06	.....	...	
		Do.	Nymphs ?	4th Nov. '06	12th Nov. '06	8 ?	Died 15th November 1906. Piroplasma in blood.
No. 4	111	Do.	Larvæ	27th Oct. '06	.....	...	
			Nymphs	4th Nov. '06	8th Nov. '06	4 ?	Died. Piroplasma in blood.
No. 4	112	Do.	Larvæ	27th Oct. '06	.....	...	
			Nymphs	4th Nov. '06	8th Nov. '06	4 ?	Died. Piroplasma in blood.
No. 4	113	Control	.....	.....	.....	...	Remained free from infection until destroyed on 16th November 1906.

TABLE III.—*Shewing in tabular form the results of feeding experiments*—contd.

Litter.	Number of Dog.	Species of tick.	Stage of tick.	Date on which ticks were added to cage.	Date on which infection was first noticed.	Incubation period in days.	Remarks.
No. 6	118	R. bursa . .	Nymphs and adults	.....	.....	...	No infection resulted.
No. 6	119	Do. . .	Do.	.....	... ..	...	Ditto.
No. 7	120	R. sanguineus	Larvæ . .	28th Oct. '06 .	.....	...	
			Nymphs . .	4th Nov. '06 .	9th Nov. '06 .	5 ?	Died. Piroplasma in blood.
No. 8	121	Do. .	Do. . .	13th Nov. '06 .	17th Nov. '06 .	4	Died. Piroplasma in blood.
No. 8	122	Do.	Nymphs? . .	13th Nov. '06 .	.....	...	
			Adults . .	22nd Nov. '06 .	28th Nov. '06 .	6 ?	Died. Piroplasma in blood.
No. 10	126	Control . .	.....	.....	.....	...	Kept from 27th October 1906. Destroyed after some weeks. No piroplasma.
No. 11	127	R. sanguineus .	Adults . .	29th Nov. '06 .	4th Dec. '06 .	5	Recovered. Piroplasma in blood.
No. 11	128	Control . .	.....	.....	.. ...	...	No infection resulted.
No. 11	129	R. sanguineus .	Adults . .	29th Nov. '06 .	4th Dec. '06 .	5	Piroplasma in blood.
No. 11	130	Control . .	.....	.....	.....	...	No infection resulted.
No. 12	131	R. sanguineus .	Adults . .	11th Dec. '06 .	21st Dec. '06 .	10	Piroplasma in blood.
No. 12	132	Do. .	Do. . .	11th Dec. '06 .	21st Dec. '06 .	10	Ditto.
No. 14	135	Do. .	Do. . .	10th Dec. '06 .	18th Dec. '06 .	8	Dying state when first observed. Piroplasma.
No. 14	136	Do. .	Do. . .	10th Dec. '06	18th Dec. '06 .	8	Blood swarming with piroplasma when first observed.
No. 15	137	Control . .	.....	.....	.....	...	From 1st December 1906. Remained throughout experiments free from infection.
No. 15	138	Do. . .	.....	.....	.....	...	Ditto ditto ditto.
No. 15	139	R. sanguineus .	Adults . .	10th Dec. '06 .	18th Dec. '06 .	8	Swarming with piroplasma when first observed.
No. 15	140	Do. .	Do. . .	10th Dec. '06 .	18th Dec. '06 .	8	Ditto ditto ditto.

V.

DEVELOPMENTAL CYCLE OF PIROPLASMA CANIS IN THE TICK.

Trustworthy observations upon developmental stages of piroplasma in the tick prior to those of Koch (188 and 189) do not, so far as I am aware, exist. Koch notes that in the gut of certain ticks *R. australis*, *R. evertsi* and *H. ægyptium*, *Piroplasma bigeminum* increases in size and develops long ray-like processes. Later on certain of these star-shaped forms are seen associated in couples, an association which Koch considers may be a kind of conjugation. Eventually the processes are retracted and the bodies assume a globular shape. Koch also describes and figures some other stages, notably masses of young amœboid parasites and, in the egg, certain large club-shaped forms four times the size of piroplasma in the blood.

A similar formation of star-shaped forms is also noted by Koch in the case of the minute parasite of East Coast fever, in this case only in the tick *R. australis*.* The earlier portion of this development is described by Kleine as taking place *in vitro*. My own observations, however, confirm this only in a very modified way, it being very doubtful whether the formation of processes such as those described by Koch and Kleine is an essential part of development.

Though these facts are a considerable advance upon previous knowledge they do not enable one to assign any particular cycle of development to piroplasma; and the relation of the club-shaped bodies to the other stages is quite indefinite.

In a preliminary note (185) I described in addition to those just mentioned certain other developmental forms of piroplasma and shewed that development eventually resulted in swarms of bodies somewhat resembling piroplasma as seen in the blood and that these were congregated in the salivary gland of nymphs of the second generation.

Unfortunately at the time of writing this note I was unaware that infection could be transmitted by *R. sanguineus* not only hereditarily, but directly from the nymph to the adult, and that in some cases I had been dealing with nymphs shewing both forms of infection. A realisation of this fact made it apparent that the developmental cycle was in reality much simpler than I had supposed, and in the present memoir I am able to give with some degree of certainty at least the outlines of development of the parasite, and to indicate

* *Vide*, however, Lounsbury's and Theiler's experiments on the transmission of this parasite.

the main facts regarding the mechanism by which infection is transmitted both in hereditary and in stage to stage infection.

The technique used has been based mainly on a knowledge of the anatomy of the tick. The principal features of this have been given by me in a previous publication and some further points which have an importance in relation to the development of piroplasma have already been mentioned in this Memoir (latter portion of Part III).

Gorged females are examined by partial dissection and the removal of portions of the gut or of the ovary and oviduct for examination fresh or after the preparation of films. In fresh preparations the club-shaped forms are generally to be found without difficulty, once their general appearance is known, in or about the ovary and oviduct, especially if a low power (1/6 objective) is used for their detection.

Developmental forms in the gut of the adult are usually more difficult to obtain. It is probable that this portion of the development takes place rather quickly and that the parasites have at the period of full engorgement already left many of the diverticula. The best results have been obtained by removing partially fed adults at a time when, by a series of examinations, it is known that the dog has passed through a period of intense infection immediately before.

Preparations shewing the tissue of the developing adult have been obtained by cutting off with a sharp scalpel the anterior third of the gorged nymph at various periods after it has dropped from the host, and after turning out the embryonic tissue making squash preparations in the usual way.

Such a procedure enables one to avoid the gut contents which remain almost entirely in the discarded posterior portion of the nymph.

Formation of the club-shaped bodies.

In preparations of the gut of ticks fed on dogs whose blood has contained numerous parasites the early stages of development of the parasite are seen. This development occurs in parasites not distinguishable from ordinary free forms,* which increase considerably in size becoming globular bodies 4 to 5μ in diameter. In the largest forms an achromatic line appears which divides the parasite into two nearly equal segments; only one of the segments contains chromatin, an appearance which was wrongly interpreted by me in my earlier note as being of the nature of conjugation (fig. 4, 3). The two portions marked off by the achromatic line eventually separate from one another except at one point, and the portion devoid of chromatin swings round to form the tail-piece of the club-shaped body, the rest of the parasite becoming the front

* Like myself, Nuttall and Graham Smith have failed to detect any forms of the parasite which there seems any reason to regard as "gametes."

half of this structure (fig. 4, No. 4). After the separation of the tail-piece there are often irregular jagged processes and the parasite appears as shewn in Pl. I, fig. 56, but later all inequalities become smoothed off and a perfect but immature club-shaped body results. Shortly after being formed the club-shaped bodies leave the gut.

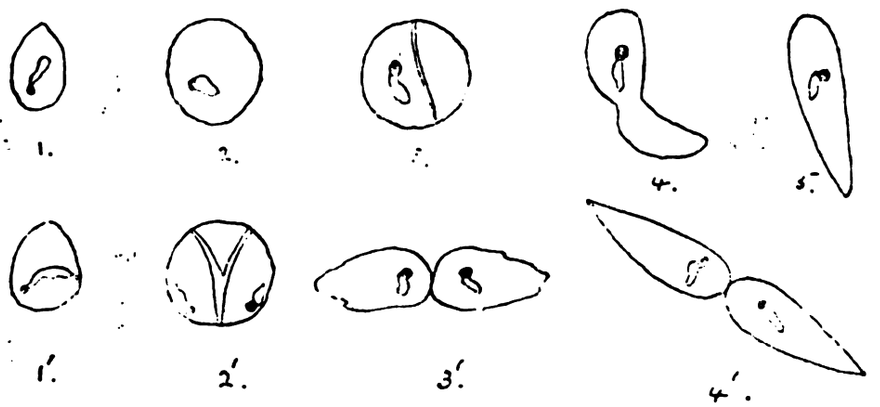

FIG. 4.

Shewing origin of single and double club-shaped bodies.
1—5. Formation of single club-shaped bodies.
1'—4.' Formation of double club-shaped bodies.

Fig. 1. Parasite undergoing enlargement.
Fig. 2. Enlarged parasite before the appearance of achromatic line.
Fig. 3. Parasite with achromatic line marking off future tail-piece.
Fig. 4. Separation of tail-piece.
Fig. 5. Fully formed club-shaped body.
Fig. 1'. Parasite in early stages of fission enlarged.
Fig. 2'. Round body with complicated achromatic lines which indicate the line of fission producing two bodies as shewn in fig. 3'.
Fig. 3'. Double body.
Fig. 4'. Double body in which each half has developed into a club-shaped body.
The area of chromatin indicated by the single line is the representative of the extension seen in piroplasma in the blood.

If the formation of long processes really takes place as described by Koch and Kleine it must be in the early stages during which the parasite is increasing in size, prior to the separation of the tail-piece. Parasites exhibiting some such appearance certainly do occur, but as a rule some irregularity of outline only is noticed, and in many cases development seems to take place without any processes at all being formed. Whether the forms figured in Pl. I, fig. 48, which resemble certain forms figured by Koch, have any particular significance I am unable to say.

As a rule parasites shewing development are found lying by themselves, either in the gut contents, or, as it would sometimes appear, embedded in the protoplasm of a cell of the gut. At other times large groups of forms are

encountered as shown n Pl. I, fig. 53. The occurrence of these large groups at first led me to suppose that in the gut the parasite underwent fission, but I have since come to the conclusion that such groups are merely the outcome of development of a cluster of original parasites. In support of such a view is the fact that double forms and groups of four are also common. Even club-shaped bodies are frequently seen in pairs or in a group of four closely situated forms (Pl. II, figs. 13 and 12).

In addition to the ordinary forms there are certain very peculiar bodies possessing a double chromatin mass and shewing achromatic lines (Pl. i, fig. 52). Koch has figured a body of this nature (*vide* his Pl. II, fig. 24) and considers it a stage in development of the parasite. They appear to be merely instances where a parasite on the point of division has been taken into the gut and has proceeded to develop. The achromatic lines have the same significance as in the single forms, but indicate in this case the lines of cleavage in the formation of two club-shaped bodies, which at this stage are short stumpy forms as shewn in fig. 4.3'. The results of division of these forms are usually shuttle-cock-shaped bodies (Pl. I, figs. 54 and 55). They are often seen still connected by their bases so as to form double forms, but many are seen lying free.

The double forms certainly at first sight suggest some kind of conjugation and it is almost certain they are the forms described by Koch, though they do not possess the sharp ray-like processes described by this author. Each parasite, however, develops into a club-shaped body and it is evident that the single forms are the result of separation of the double forms and not *vice versâ*. The method of formation of these bodies and of the ordinary club-shaped forms is diagrammatically represented in the accompanying figure.

In my preliminary note I described club-shaped bodies as occurring in hereditarily infected nymphs and I was puzzled to account for the persistence or recurrence of these forms until it became clear that they arose merely from a reinfection of the nymphs from dogs which had become infected as a result of the bites of other nymphs of the same brood. This complication it is easy to understand, since there would be a considerable interval between the time of attachment of the first and last nymphs of a batch, an interval more than sufficient to bridge over the short incubation period which was present in these experiments.

As the result of feeding nymphs on infected dogs it was discovered that the same development of the parasite takes place in the gut of this stage as in the gut of the adult and, as in the latter case, results in the formation of club-shaped bodies, each club-shaped body being the direct representative of an original parasite in the blood. Since these club-shaped bodies are found to go through

development and, as described later, to form sporozoits in the salivary glands of the adult, it is practically certain, though not yet proved by actual experiment, that in *R. sanguineus* infection taken in by the nymph can be transmitted by the adult.

Whether any sexual process is involved in the formation of the club-shaped bodies cannot yet be definitely stated; but there are sometimes seen parasites in which the chromatin is arranged in the form of granules scattered over the surface, and in one instance a very significant appearance was seen where a smaller pear-shaped form had apparently separated itself from such a body.

The method of development in the tick certainly suggests a sexual cycle, and it is immediately prior to the formation of the club-shaped bodies that one would expect the junction of the male and female elements to occur. As just stated, the double forms are not conjugation forms and the analogy of development of piroplasma with that of the malarial parasite would lead one to suppose that the male element is small, and in such a situation as the gut of the tick likely to be easily overlooked. The absence of any definite observations regarding fertilisation is not therefore sufficient at this stage to negative the view that the cycle of development in the tick is a sexual one.

Club-shaped bodies.

These bodies whose origin we have just described are found in the gu of the adult tick fed on infected dogs, and in still greater numbers about the ovaries and oviducts, and in the ova. In nymphs fed on infected dogs they are found in the gut and in the tissues.

There is no appreciable difference between the bodies as seen in the adult and the nymph, and their origin in both cases is obviously from a parasite taken into the gut of either of these forms.

They are most easily detected in fresh preparations and are visible even under a 1/6 dry objective. Under a high power two types are seen.

(*a*) Rather rigid thorn-like bodies resembling on a very small scale certain gregarine trophozoits. This resemblance is largely due to the presence of a peculiar disc-like structure armed with cusps carried at the anterior extremity. They are sometimes motionless, but usually exhibit rather slow movements, especially a side to side flap-like action of the tail portion. The protoplasm is transparent, slightly refractile, and free from large granulations, and in it a clear area (the nucleus) can often be made out.

The disc has the appearance of a boring organ. It carries four or five cusps, one of which is situated centrally, whilst the others are arranged round the periphery. Immediately behind this structure the parasite is often constricted so that a kind of neck is formed.

(*b*) Leech-like forms more club-shaped than those just described and executing very active movements. They possess a swollen end which is often attached to the slide or cover glass and a thin end which is kept in constant motion like the head of a leech. They also undergo modified amœboid movements, the shapes depicted in Pl. II, fig. 2, being characteristic.

These bodies are most numerous as a rule about the oviducts, and a few can often be found here when not to be detected elsewhere. They also occur in the ovary and may be seen lying within ova not yet set free in the lumen, and in more mature ova. By careful search they can also be found in fresh preparations of the gut.

In stained preparations a greater variety of forms is apparent, but the two forms mentioned, those with and those without a disc, are distinguishable. Since intermediate stages are not difficult to find it is probable that both are identical in nature, the disc bearing forms being the more mature stage.

The protoplasm of the club-shaped forms is finely granular and stains a light blue with Giemsa stain. It is often homogeneous but denser towards the periphery; very frequently it is vacuolated. The vacuoles when present are large irregular non-refractile spaces occurring, as a rule, on either side of the chromatin mass which most frequently lies in a portion of protoplasm free from vacuoles. There is sometimes a darkly staining spot at the tail end, and a small spike described by Koch is sometimes visible. Appearances suggesting that the parasite has a leaf-like form are common, but may be the result of preparation of the specimen. A common appearance in the less mature forms is a constriction about the middle of the parasite due to the method in which the body is formed.

The chromatin consists in the younger forms of a dense mass with a lightly staining extension, lying most frequently at right angles to the parasite as it often does in piroplasma in the blood; at other times the extension is directed towards the pointed end or even anteriorly, a position which it assumes immediately prior to the formation of the disc. In the mature club-shaped forms there is as a rule no extension, the chromatin consisting of a single dense mass surrounded by a pale achromatic area. It is circular or irregular in shape and is often star-shaped with sharp ray-like processes.

The disc and very often a portion of the neck may stain red like the chromatin or a dark purple. It shews very often an achromatic line around its circumference. The first indication of the formation of the disc is the passage forwards of a portion of the chromatin which is at first attached by a filament to the main mass but later becomes separated (Pl. II, fig. 10). The separated portion of chromatin then divides and eventually becomes diffused throughout the anterior end of the parasite. Later on the anterior extremity of the parasite

stains a deep purple and the chromatin appears to have disappeared. Many forms appear to proceed to develop into zygotes even without having possessed a disc.

Double club-shaped forms are commonly seen. They originate from the double forms already described, in which case they are attached by the broad end, or they may occur by fission of certain very stout club-shaped forms in which case they are generally arranged alongside one another (Pl. II, figs. 15 and 17).

As already noted the club-shaped bodies leave the gut and are found in the ovary and oviducts of the adult or in the tissues of the nymph.

In both situations they undergo changes, becoming converted into globular or oval bodies having a characteristic appearance which I have for convenience of description and on account of its analogy with this stage in the malarial parasite called the "zygote."

The change of the club-shaped body into the zygote can be followed, forms being seen in which the thicker end of the club-shaped body has become swollen whilst the tail end is still recognisable (Pl. II, figs. 18 to 21), or in which the body of the club-shaped form is swollen so as to form a globular mass, but both the head and tail end are still traceable. In some cases a dark area, the remains of the disc, is still evident on the periphery of the young zygote.

The Zygote.

The zygotes when first formed are oval or round bodies in which the protoplasm stains a dark blue with Giemsa stain and the chromatin of which at first resembles that of the club-shaped bodies. They generally lie within a cell though they are frequently seen free, possibly the result of rupture of the cell in the making of the preparation. In the ovum they lie in the yelk, but in the nymph they lie embedded in the protoplasm of a cell, two and even three zygotes sometimes being present in a single cell. In some instances the zygote lies in a space in the cell, but more usually they are embedded in the protoplasm so that their outline is seen only on careful examination. In most cases the young zygote is more or less globular, but not infrequently they seem to have processes extending into the protoplasm of the host cell. They increase greatly in size, becoming very large somewhat irregular bodies as much as 25 μ in diameter. The chromatin, at first identical with that of the club-shaped bodies, increases greatly in amount and sends out long filaments into the protoplasm. After a time the chromatin has formed two and later on as many as half a dozen main masses, and from these extend a plexus of chromatin filaments, many of them ending near the periphery of the parasite n a slightly clubbed extremity.

The eventual fate of the zygote is to break up into sporozoits, though owing to certain conditions shortly to be described the nature of this process often becomes obscured. In a previous note (185) I have described the formation of certain bodies which I called "sporoblasts" from fission of a single parent form and another cycle characterised by large "sporulating" or "rosette" forms. Further investigation has shewn that the appearances seen were due merely to variations in the method of breaking up of the zygote.

In some cases the zygote breaks up in a very regular fashion, numbers of sporoblasts being arranged in a rosette (Pl. II, fig. 24). This may go on to the formation of sporozoits in which case an immense number of small forms are arranged as in Pl. II, fig. 26.

More commonly the zygote forms a loose group of very characteristic "sporoblasts" (Pl. II, fig. 29) and these later form "sporozoits." The sporoblasts are extremely characteristic of hereditary development in the larva, and of stage to stage development in the tissues of the gorged nymph. Either by their own movements or by division of the cell in which they lie they come to be disseminated through the embryonic tissue cells, but whether their growth continues after separation from the zygote is uncertain. The chromatin of the newly formed sporoblasts consists of a single or of two or more masses with filamentous extensions such as are seen in piroplasma in the blood. These extensions ultimately point to various portions of the periphery where new chromatin masses are formed. Fission then takes place and the sporoblast becomes a number of sporozoits.

In the salivary glands of nymphs some comparatively large, very regular pear-shaped forms occur possessing the reticular extension. Whether these are sporozoits or whether they are again liable to division I have not been able with certainty to ascertain, but the latter appears probable since the ultimate products of division have only a single chromatin mass. Whatever their nature they are capable of giving rise to infection in the dog. On the whole it is perhaps at present wise not to be too dogmatic regarding the significance of the immature products of the division of the zygote, it being certain that the ultimate products are the bodies here termed sporozoits.

Sporozoits.

These are small bodies about the size and having the general appearance of some of the forms of piroplasma in the blood.* They may be pear-shaped or circular, and under some circumstances they are seen with what appear to be pseudopodia extended (Pl. III, fig. 8). The protoplasm stains a

* In smears of the contents of nymphs it is easy to mistake these for blood forms if, as often happens, they become disseminated through the specimen. (*Vide* Pl. II, figs. 26, 27, and Pl. III, fig. 8.)

pale blue and often has one or two non-refractile vacuoles. The chromatin mass is single without any extension. It is situated most frequently more or less centrally, but may be at or near the periphery. Star-shaped or otherwise irregular forms have a similar structure.

The number of forms resulting from a single oocyst is very considerable, but for reasons to be given later they are rarely found all in the same situation, but infiltrate as it were the tissues (Pl. III, figs. 4 and 5).

Relation of the Parasite to the Tissues.

In the hereditarily infected larva sporoblasts are found distributed among the cells, but the exact relation which the parasite bears to the tissues has not yet been worked out, both the egg and the larva being difficult to manipulate satisfactorily. Fortunately much more has been ascertained regarding the passage of the parasite from nymph to adult.

In the nymph we have seen that parasites taken in develop into club-shaped bodies. These after leaving the gut penetrate the tissues in all directions and enter cells. The cells chosen are as a rule cells of the embryonic tissue, which is by this time forming everywhere in the body of the gorged nymph, and which I have referred to as forming within the cuticle of the nymph a rough model of the future adult tick. Not infrequently, however, the cells of the gut itself appear to be invaded and the parasite proceeds to develop in this position.

A peculiarity of the processes of cell invasion is the small amount of change set up in the victimised cell. Even when a parasite has almost replaced the protoplasm of a cell with swarms of young parasites the external contour and general appearance of the cell is retained. Such cells may quite well be mistaken by one unfamiliar with the appearances for normal tissue cells containing granules of chromatin-like substance such as some normal cells in the tick do, but they have only to be ruptured in the preparation of the specimens to demonstrate at once the parasitic nature of their contents.

We have previously seen that any given cell of the embryonic tissue at the base of the limbs stands a chance of becoming the ancestor of cells of one or more salivary acini, and the presence in such a cell of a zygote does not prevent it from carrying out its appointed task. The result is that so soon as the sporoblasts are formed they become separated by the repeated cell division and when in their turn the sporozoits are formed these are disseminated throughout a number of cells some or all of which end by being functionally active salivary cells.

Though this is the history of a large number of the parasites there are undoubtedly a great many club-shaped bodies which in the first place select cells, not the progenitors of salivary tissue. Zygotes and the products of these have

been seen in the embryonic cells of the cuticle, in tracheal and in fat cells. A very common situation for swarms of sporoblasts or sporozoits is certain cells lying about the muscle bundles (Pl. III, fig. 13). It seems impossible to believe that all these parasites perish and it is probable that by some means they eventually reach the salivary gland as do those of the malarial parasite in the case of the mosquito. For some time after the adult tick has hatched there is still salivary tissue in process of development, and the parasite must have ample time to reach this before the tick leaves its host. That both sporoblasts and sporozoits have the power of independently invading cells is shewn by the frequency with which appearances like those shewn in Pl. III, figs. 11 and 12, are met with.

Summary and Conclusions.

In order to make quite clear what has just gone before it may be advantageous to summarise the main outlines of the course of development in the tick.

(1) In *R. sanguineus* there are two means by which infection is transmitted.

(*a*) Hereditarily through the egg. A method shewn both by experimental infection of dogs and observation of the parasite in the tick.

(*b*) Stage to stage infection. Not yet proved by experimental infection but practically certain from observations upon the parasite.

(2) In both methods of infection the parasite goes through the same cycle of development becoming in turn a club-shaped body and then a zygote which breaks up into "sporoblasts" and these again into "sporozoits."

(3) In hereditary infection club-shaped bodies originating each from a single parasite penetrate the ova either in the ovary or in their passage down the oviduct and in the yelk become zygotes. In the larva the zygotes have broken up into sporoblasts which are found disseminated in the tissues and in the nymphs the sporozoits have accumulated in large numbers in the salivary glands.

(4) In infection from nymph to adult the club-shaped bodies after being formed in the gut of the nymph penetrate cells of the embryonic tissue which will eventually form the adult, and embedded in the cells of this they become zygotes. The sporozoits derived from these zygotes may find themselves without any action on their part in salivary cells or they may be situated elsewhere, in which case they probably reach the salivary cells by their own movements, possibly aided by the circulation of fluids in the tick.

The details of development strongly suggest a cycle of a sexual nature, and if this be the case the sexual cycle of piroplasma has many points in common with the sexual cycle of the malarial parasites and proteosoma. This has been already pre-supposed by the nomenclature employed which it seems reasonable to use until further research either confirms or shews it to be untenable.

The greatest difference between the development and that of the malarial parasite occurs in the peculiar dissemination of the sporoblasts and sporozoits and the fact that the ookinete (?) comes to rest not in the gut wall but in the tissues. That the malarial zygote has no true wall of its own has been already supposed by Grassi and in this the zygote of piroplasma would bear it a resemblance. The separation of sporoblasts by the growth of the embryonic tissue and possibly by the movements of the sporoblasts themselves and the infiltrating action of piroplasma have so far as I know no parallel in malaria or other of the pathogenic protozoa. The sporozoits except that they have not the filiform shape of those of malarial parasites seem to correspond exactly to these and their eventual location in the salivary acini is exactly parallel to conditions in malaria. Though it is clear much has still to be done in following out details there can be no question that in the main the mystery surrounding the passage of piroplasma through the tick has been solved.

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EXPLANATION OF PLATES.

PLATE I.

Figures 1-8.—Early invasion forms in young dogs, characterised by large size of forms and early rupture of cells, which rarely contain more than four parasites. × 2000.

Figures 9-17.—Stages in the formation of a group of four parasites in a corpuscle. × 2000.

Figure 9.—Parasite increasing in size but still oval in shape.

Figure 10.—Parasite increasing in size and showing amœboid processes.

Figure 11.—Characteristic elongation of the parasite heralding fission.

Figures 12 & 13.—Later stages than figure 11. Note the appearance of the so-called blepharoplast.

Figure 14.—Formation of two parasites by constriction in the middle together with separation of the forms.

Figure 15.—Two parasites preparing for a second division.

Figure 16.—Two parasites repeating stage seen in figure 12.

Figure 17.—Formation of four parasites in a corpuscle.

Figure 18.—A corpuscle containing eight parasites. × 2000.

Figure 19.—A corpuscle containing sixteen parasites. × 2000

Figure 20.—A group of free forms resulting from rupture of a corpuscle containing sixteen parasites. × 2000.

Figures 21-23.—Fission by another method above. × 2000.

Figure 21.—Parasite shewing chromatin with a small amount of protoplasm forming buds.

Figures 22&23.—Later stages.

Figure 24.—Parasite intermediate in appearance between the early invasion forms and those figured in figures 25-29. × 2000.

Figures 25-29.—Round refractile forms with peripheral chromatin. × 2000.

Figure 29.—Parasite of this type (corpuscle not shewn) deeply stained and decolorised shewing main chromatin mass flattened and the irregular area in the form of a thread. × 4000.

Figures 30 & 31.—Parasites (corpuscles not shewn) shewing appearance sometimes taken by the "irregular" area of chromatin. × 4000.

Figures 32-40.—Parasites (corpuscles not always indicated) shewing appearances of irregular area, main chromatin mass, and blepharoplast under best conditions of staining (Giemsa). × 4000.

Figure 32.—Growing parasite with irregular area, but not so-called blepharoplast. The irregular area, however, shows some condensation in the neighbourhood of the place where the blepharoplast will later appear.

Figure 33.—Later stage heralding fission; note the appearance and position of the so-called blepharoplast.

Figure 34.—Parasites in which the chromatin heralds fission.

Figure 35.—Similar to figure 33, but the parasite is drawn out. This and the form shewn in figure 33 are extremely common forms.

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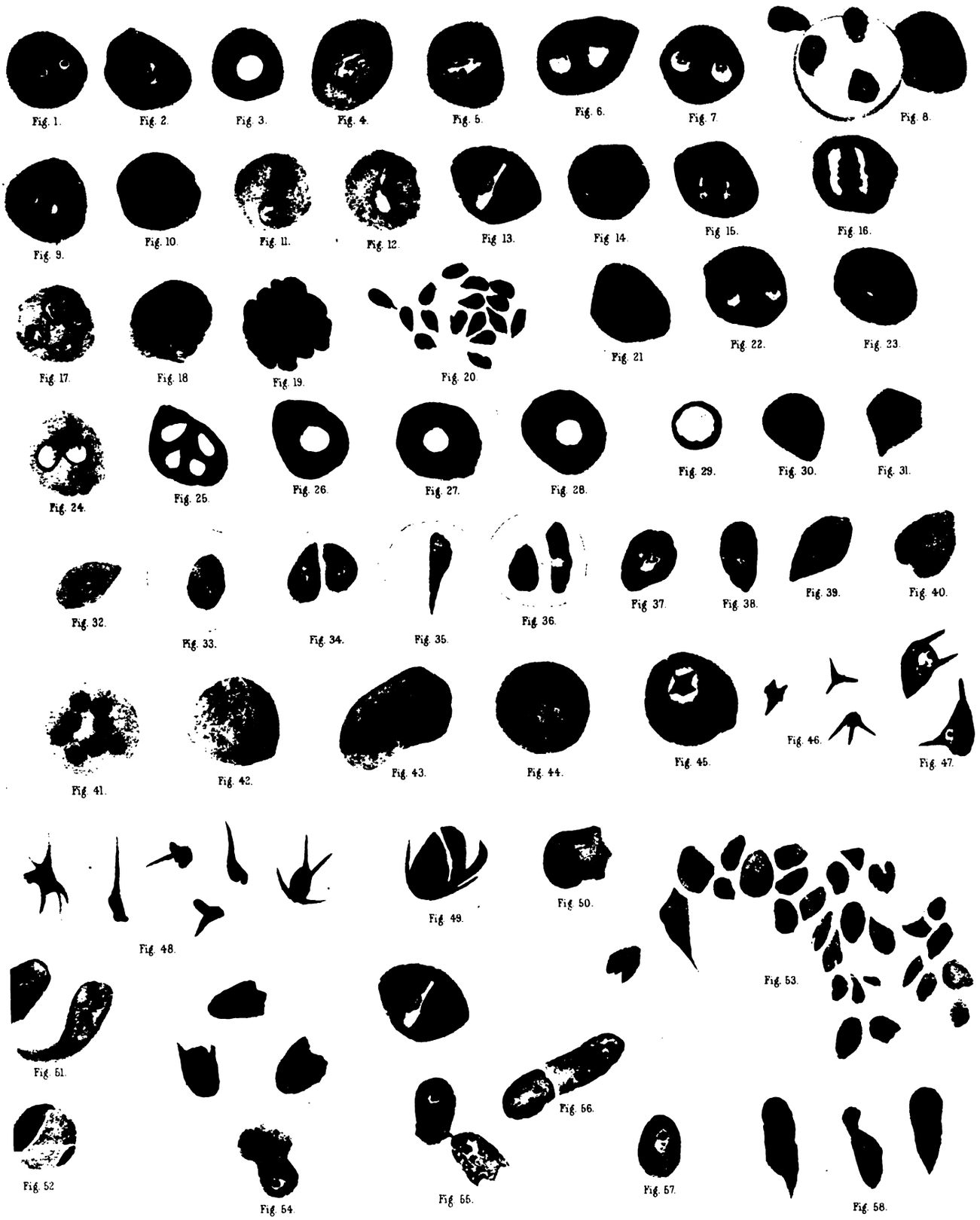

Survey of India Offices, Calcutta, July, 1907.

Figure 36.—Shewing two parasites, one elongated and the other rounded in form in the same cell and the arrangement of the chromatin.

Figures 37-40.—Free forms.

Figure 37.—Free form. Similar chromatin arrangement to figure 32.

Figure 38.—Free form with chromatin not yet shewing signs of activity.

Figure 39.—Free form in a stage heralding fission. This is a common type.

Figure 40.—Free form (early invasion type) suggesting a more direct method of fission.

Figure 41.—Parasites from heart blood after death. Note the peculiar change to small dark staining round forms such as are sometimes figured in *p. equi.* × 2000.

Figure 42.—A parasite from the heart *post mortem* shewing extrusion and apparent separation of processes. × 2000.

Figures 43& 44.—Forms seen in culture but also under certain conditions *in vivo.* × 2000.

Figure 45.—Culture form lying in corpuscle. × 2000.

Figure 46.—Culture forms free. × 2000.

Figure 47.—Large culture forms with processes. × 2000.

Figure 48.—Forms from the gut of the tick with processes. The position of the chromatin mass on the periphery or even projecting from the parasite is common. Such forms are not invariably seen. × 2000.

Figures 49&50.—Peculiar forms, stages in the production of the club-shaped bodies. As the plate was prepared before the true nature of these bodies was apparent, the forms are not well selected to shew formation of the clubs (see figure in text). × 2000.

Figure 51.—Oval and club-shaped forms from the gut of adult tick. × 2500.

Figure 52.—Double form with achromatic lines, early stage of so-called "copulation" forms. × 2000.

Figure 53.—A group of developing parasites in the gut of adult tick. Such forms develop each into a single or more rarely a double club-shaped body. × 1000.

Figure 54.—Bodies resulting from separation of the so-called "copulation" forms. In two the chromatin is seen, sending out a process probably the rudiment of the disc (see Plate II, figures 10 and 11). × 2000.

Figure 55.—Double form so-called "copulation" bodies but apparently merely the result of development of a parasite which was on the point of division when taken into the tick, (see figure 52, also figure on page 57). × 2000.

Figure 56.—The same but shewing an elongated form. × 2000.

Figure 57.—A developing club-shaped body. × 2000.

Figure 58.—Club-shaped bodies soon after their formation. × 2000.

PLATE II.

Figure 1.—Wall of oviduct, two spermatozoa (sp.) and three club-shaped bodies (p.) Fresh preparation 1/6 objective.

Figure 2.—Club-shaped bodies from the ovary. Fresh preparation. The upper figures are mature disc-bearing forms, the lower are leech-like forms shewing euglenoid movements. × 2000.

Figure 3.—Club-shaped bodies without discs. × 2000.

Figures 4 & 5.—Club-shaped bodies with cusp-bearing discs. × 2000.

Figure 6.—Club-shaped body with disc seen on the flat. × 2000.

Figure 7.—Club-shaped body with disc seen on the flat and the body folded upon itself. × 2000.

Figure 8.—A lub-shaped body shewing vacuoles and diffuse reddish staining of anterior portion. × 2000.

Figure 9.—Club-shaped bodies similar to those seen in the lower part of figure 2. × 2000.

Figure 10.—Club-shaped body shewing process of chromatin passing forwards to form disc. × 2000.

Figure 11.—Later stage in the disc formation. × 2000.

Figure 12.—Later development of a double form in which the component parts have not yet separated. × 2000.

Figure 13.—Three of four forms seen in close connection. × 2000.

Figure 14.—Small club-shaped body. × 2000.

Figure 15.—Double club-shaped body resulting from fission of large fat club forms,—a common appearance but the significance of which is not known. × 2000.

Figure 16.—Unusual form of club-shaped body. × 2000.

Figure 17.—Club-shaped body apparently with double chromatin mass, but there are really two club-shaped bodies as in figure 15. × 2000.

Figure 18.—Club-shaped body in yelk in process of changing into a zygote. Note the presence of the "tail" and the dark area on the periphery corresponding to the disc. × 2000.

Figure 19.—Further stage than above but still shewing situation of the "tail" and disc. Note also the stellate character of the chromatin seen also in figure 8. × 2000.

Figure 20.—A young zygote in an embryonic cell. Note the commencing extension of the chromatin and the more darkly staining nature of the protoplasm. See also figure 7, Plate III. × 2000.

Figures 21-23.—Growing zygotes. Note the peculiar processes of the chromatin, of which, owing to their delicacy and complexity, the uncoloured figures give an imperfect idea. × 2000.

Figure 24.—A zygote breaking up so as to give rise to the appearance of a "sporulating" body. In this as in the next figure the pale blue protoplasm and the characteristic chromatin are poorly shewn. Specimen from embryonic tissue. × 2000.

Figure 25.—A mass of imperfectly divided "sporoblasts" which have replaced the protoplasm of an embryonic cell the nucleus of which is visible. Compare figures 26 and 27 which is the ultimate product of such a condition. × 2000.

Fig. 1.

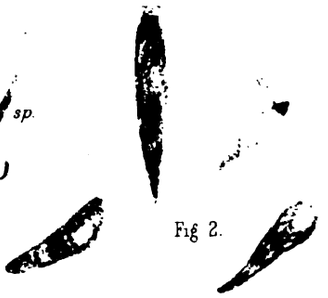
Fig. 2.


Fig. 3.


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Fig. 6.


Fig. 7.

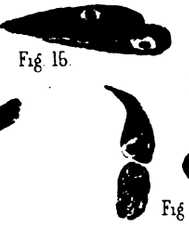

Fig. 8.


Fig. 9.


Fig. 10.


Fig. 11.


Fig. 12.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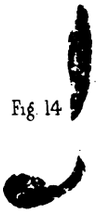
Fig. 14.


Fig. 16.


Fig. 17.


Fig. 18.


Fig. 19.


Fig. 20.


Fig. 21.


Fig. 22.


Fig. 23.

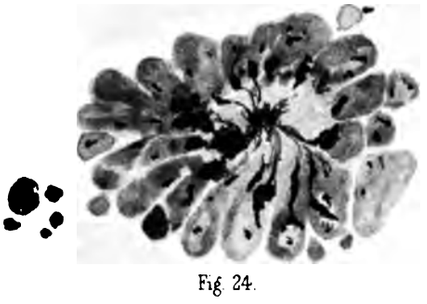
Fig. 24.

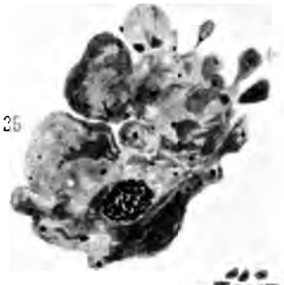
Fig. 25.

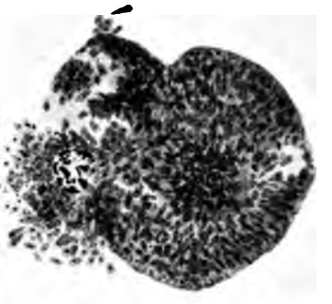
Fig. 26.


Fig. 37.

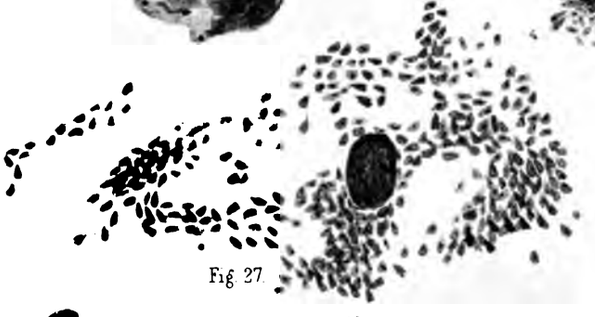
Fig. 27.


Fig. 28.


Fig. 29.


Fig. 30.

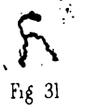
Fig. 31.


Fig. 32.


Fig. 33.


Fig. 35.

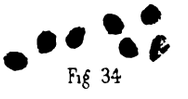
Fig. 34.

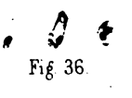
Fig. 36.

Figure 26.—An embryonic salivary cell of future adult the substance of which is replaced by sporozoits. To the left is seen the nucleus of the cell, to the right a globular mass—a later stage than that shewn in figure 24.

Figure 27.—An embryonic cell squashed out shewing the swarms of young parasites ("sporozoits?" × 1000.

Figure 28.—Some of the above more highly magnified. × 2000.

Figure 29.—A group of "sporoblasts" shewing that they are about to divide further into "sporozoits." From embryonic tissue. × 2000.

Figure 30.—A group of bodies seen in the salivary cells of hereditarily infected nymphs. They appear to be capable of further subdivision by the process indicated in figures 29, 31 and 33. Note the resemblance of the chromatin to plroplasma in the blood and the presence of the irregular area. × 2000.

Figure 31.—A "sporoblast" shewing extension of chromatin very like that in piroplasma. Such a body will eventually form three parasites. × 4000.

Figures 32 & 33.—Later stages of the condition seen in figure 31. × 2000.

Figure 34.—Sporozoits from salivary cells. × 2000.

Figure 35.—Angular and stellate sporozoits—the chromatin has from an overight not been indicated. × 2000.

Figure 36.—Pear-shaped sporozoits exactly resembling piroplasma in the blood. From salivary gland. × 2000.

Figure 37.—A group of four club-shaped bodies in the gut of nymph fed on infected dog. × 2000.

PLATE III.

Figure 1.—Portion of undeveloped salivary gland of adult showing the large clear "poison" acini and the more retractile and granular ordinary acini. x 1/3 objective.

Figure 2.—A developing acinus. Ordinary type. × 1000.

Figure 3.—A developing acinus, "poison" acinus. Note the large cell which in this type of acinus develops out of all proportion to the rest of the acinar cells. × 1000.

Figure 4.—Embryonic adult tissue the cells of which contain sporozoits. The dark mass represents the original sporulating zygote, the two large light cells are becoming salivary tissue. × 750.

Figure 5.—Embryonic tissue infiltrated with the products of division of zygote. Note on the right some free "sporoblasts." × 750.

Figure 6.—A salivary cell of a nymph (a portion only is shewn) containing sporozoit-like bodies. Note the close resemblance to pyroplasms in the blood the irregular area of chromatin, etc. The granular protoplasm and secretory products have been omitted. × 2000.

Figure 7.—A young zygote embedded in embryonic salivary tissue cell. Note to the right and above the rudiments of a duct with one of its nuclei × 1500.

Figure 8.—A squash preparation of embryonic salivary tissue of adult shewing sporozoits. × 2000.

Figure 9.—Two poison acini (developing) containing sporozoits. The lower right hand space is a portion of the acinus shewn above which is overlain in the middle by the duct. × 750.

Figure 10.—Sporozoits in embryonic tissue cell shewing that when the cell divides there will be two infected cells. The sporozoits seen are probably the progeny of only one or two sporoblasts. × 1500.

Figure 11.—Single sporozoits in embryonic salivary cells. × 2500.

Figure 12.—A group of sporozoits resulting from one or at most two sporoblasts. Embryonic salivary tissue. × 2500.

Figure 13.—A mass of sporozoits occupying substance of a cell of muscle sheath. × 1000.

PLATE III.

Figure 1.—Portion of undeveloped salivary gland of adult shewing the large clear "poison" acini and the more refractile and granular ordinary acini. 1/3 objective.

Figure 2.—A developing acinus. Ordinary type. × 1000.

Figure 3.—A developing acinus, "poison" acinus. Note the large cell which in this type of acinus develops out of all proportion to the rest of the acinar cells. × 1000.

Figure 4.—Embryonic adult tissue the cells of which contain sporozoits. The dark mass represents the original sporulating zygote, the two large light cells are becoming salivary tissue. × 750.

Figure 5.—Embryonic tissue infiltrated with the products of division of zygote. Note on the right some free "sporoblasts." × 750.

Figure 6.—A salivary cell of a nymph (a portion only is shewn) shewing sporozoit-like bodies. Note the close resemblance to piroplasma in the blood the irregular area of chromatin, etc. The granular protoplasm and secretory products have been omitted. × 2000.

Figure 7.—A young zygote embedded in embryonic salivary tissue cell. Note to the right and above the rudiments of a duct with one of its nuclei × 1500.

Figure 8.—A squash preparation of embryonic salivary tissue of adult shewing sporozoits. × 2000.

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Fig 1.

Fig 4.

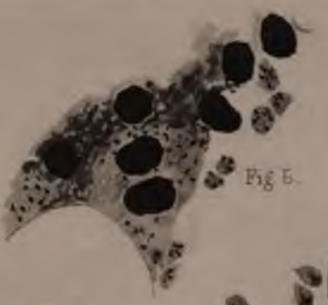


Fig 5.

Fig 2.

Fig 3.

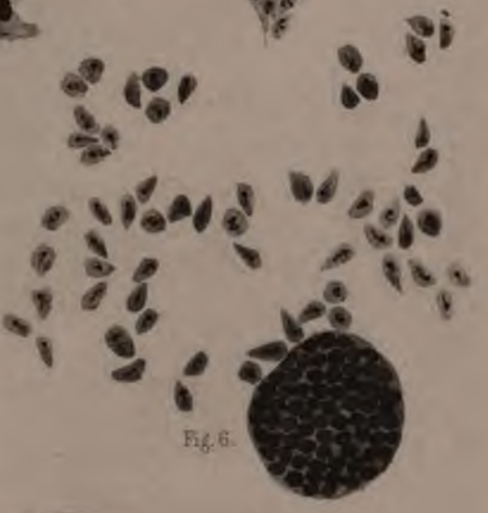


Fig 6.

Fig 7.

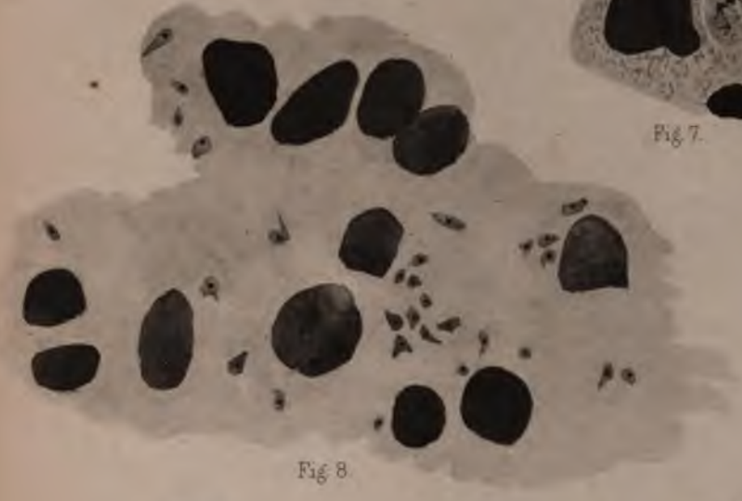
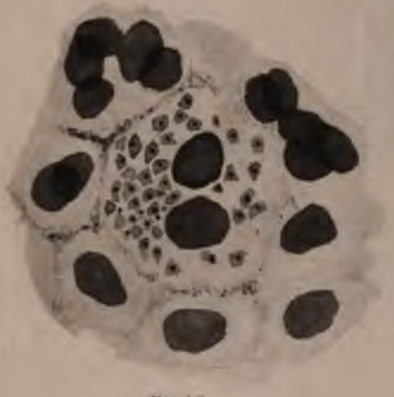


Fig 8.

Fig 9.



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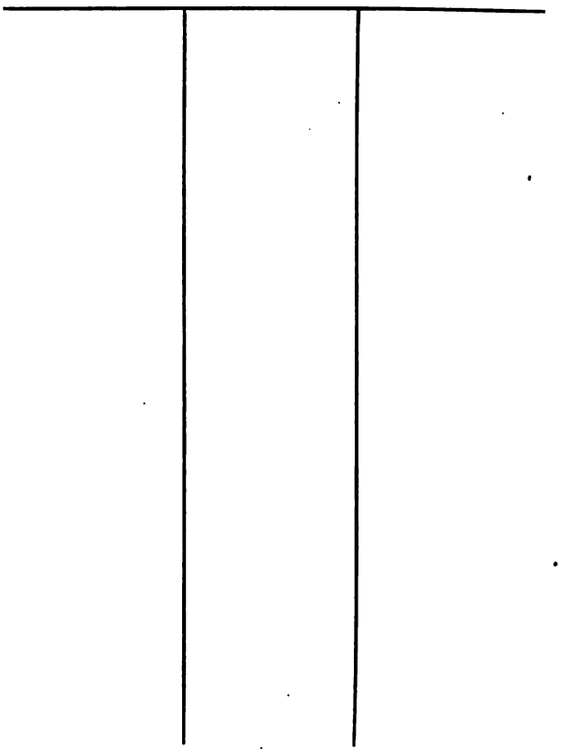

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